Aspects of the conservation biology of *Coregonus lavaretus* in Britain

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ABSTRACT

Conservation of phenotypically variable taxa such as the European whitefish (*Coregonus lavaretus*) can be particularly challenging. In this thesis it is argued that the recent designation of seven native *C. lavaretus* populations as three endemic species (*C. clupeoides*, *C. stigmaticus* and *C. pennantii*) by Kottelat & Freyhof (2007) are incorrect and cannot be substantiated with the results presented here. However, evidence for important infra-specific variation between populations has been found. Two native Scottish populations of *C. lavaretus* show considerable variation in morphology, trophic ecology and life history.

The variation in these populations warrants protection, one conservation action becoming more commonly utilised in Britain is conservation translocation. It was found that there were significant differences between source and refuge populations in Scotland. The wisdom of using this conservation measure on a phenotypically plastic organism is discussed. Nevertheless the establishment of further refuge populations are considered to be a viable conservation action.

Sub-structuring within the largest native Scottish population of *C. lavaretus* was not found. However, evidence of residence within certain basins of Loch Lomond was found through significant differences in muscle stable isotope signatures. Investigation was also made into the trophic ecology of other fish in Loch Lomond. It was found that brown trout (*Salmo trutta*) in Loch Lomond have a non-typical migration pattern and invasive ruffe (*Gymnocephalus cernuus*) now form an important part of the trophic ecology of this site.

In Britain several whitefish populations have been invaded by ruffe, a species native to Britain, but not to these sites. An experiment is conducted into the protective ability against ruffe predation on *C. lavaretus* ova of substrates typical on spawning grounds. It was found that pebbles and gravel form the best spawning substrate. The impact this mortality may have on the life history of Loch Lomond *C. lavaretus* is discussed.

Using information gathered in this study, recommendations for the management of *Coregonus spp*. are summerised. There is the potential for these recommendations to apply to other phenotypically plastic species that vary between sites such as Arctic charr (*Salvelinus alpinus*) and brown trout.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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AUTHORS DECLARATION

The material presented in this thesis is the result of research conducted between October 2005 and September 2009, under the supervision of Prof. Colin Adams and Dr. Colin Bean. This work has not been submitted as part of any other degree and is based on individual research carried out by myself. Any published or un-published material not of my own has been acknowledged in the text.

Chapter 1 GENERAL INTRODUCTION

1.1 TAXONOMY AND CONSERVATION

Biological conservation is the act of protecting biota and habitats from loss or extinction. This can take several forms including legal protection, *in situ* management such as habitat improvement, or *ex situ* management such as translocations. Translocation is the movement of individuals to another area, for instance to supplement another population, reintroduction to a site of previous occurrence, or movement to a new area, in conservation often termed a 'refuge' site (Griffith *et al.,* 1989; Stockwell *et al.,* 1996). This is becoming an increasingly common conservation measure, and as such guidelines for translocations have been set out by various authorities (e.g. the IUCN Position Statement on Translocation of Living Organisms, 1987; the IUCN Guidelines for Re-introductions, 1995; JNCC Policy for Conservation Translocations of Species in Britain, 2003). However, in order to effectively conserve and manage biodiversity, the groups that are to be conserved must be identified.

Taxonomy is concerned with the identification of biological groups (taxa), and their relationships to one another based on common ancestry (systematics). Nomenclature simply deals with the correct naming of taxa, however, since a species name says something about its evolutionary history and relationship to other organisms, it can also be important. Ideally a known taxonomy should be a prerequisite for the planning of conservation and management of any organism (Rojas, 1992; Samper, 2004).

The most widely used biological unit is the species (Green, 2005; Mace, 2004). This taxonomic grouping is recognised politically, in legislation and by both scientists and non-scientists. Moreover for many biota it is a clear mechanism for biological grouping. A 'good' species can be defined as one that is substantially reproductively isolated from other species (Coyne & Orr, 2004). Despite the heavy reliance on the species as a pragmatic conservation tool there are some theoretical and practical difficulties associated with its use. There are many species concepts, and at least 22 different definitions of what constitutes a species in current use (Mayden, 1997; Adams & Maitland, 2007), but not all species concepts are easily applicable. For 'difficult' biota, there continues to be significant theoretical difficulty in achieving a conceptual consensus of exactly what defines a species (Mayden, 1997; Coyne & Orr, 2004).

Only relatively recently have molecular methods become applicable to understanding evolutionary relationships (see Teletchea, 2009 for a recent review). Traditionally, taxonomy was (and often still is) based only on phenotypic traits. The phenotype is all aspects of an organism, other than its genotype (West-Eberhard, 1989). Traits may be similar due to a shared ancestor (homology) in which case they are useful in systematic taxonomy, or due to convergent evolution in a comparable environment (homoplasy). Distinguishing between the two can be a problem, particularly in closely related taxa at small taxonomic scales. There is the added possibility of phenotypic plasticity in these traits. This is plasticity in aspects of the phenotype as a response to environmental (biotic or abiotic) pressures (West-Eberhard, 1989). Thus phenotypic variation in organisms can be due to expressed variation in the genotype, or variation in the environment. Distinguishing these in nature can be difficult, however, if phenotypically different populations are raised in a common environment (a 'common garden') and the phenotypic differences are not eroded, these are due to genetic differences. Usually however, phenotypic variation is a complex mix of the two.

1.2 PROCESSES INFLUENCING VARIATION BETWEEN AND WITHIN POPULATIONS

Variation between or within populations is sometimes (but not always, Magurran, 1998) a precursor to speciation, the process that explains the origination of species. There are two main scenarios as to how speciation can occur. Allopatric speciation in which gene flow between populations is interrupted by a physical barrier e.g. geographical isolation. Populations diverge in isolation and at some point reproductive isolation is developed (Jordan, 1905; Dobzhansky, 1937; Mayr, 1963). An alternative scenario is sympatric speciation in which speciation occurs within interbreeding populations without any physical barrier to gene flow (Darwin, 1859; Johnson & Gullberg, 1998; Dieckmann & Doebeli, 1999). Speciation in sympatry is less intuitive than that in allopatry, may be less common (Futuyma & Mayer, 1980; Coyne & Price, 2000) and faces greater restrictions (i.e. through recombination) (Coyne, 2007). Nevertheless this has been demonstrated, for instance in plants (Otto & Whitton, 2000; Savolainen *et al.*, 2006), and some fish e.g. cichlids (Schliewen *et al.*, 1994; Kirkpatrick, 2000; Barluenga *et al.*, 2006). Variation within and between populations may also be due to the introgression of allopatrically developed groups that have not acquired full reproductive isolation. It has been suggested that this is responsible for the large amount of phenotypic variation in some taxa (Svärdson, 1970; Dowling & Secor, 1997; Salzburger *et al.*, 2002), although introgression and sympatric speciation may be hard to distinguish (Coyne, 2007).

Phenotypic variation due to genetic differences may be influenced firstly by effects that are random with respect to local selection pressures such as founder effects: the initial genetic variation contained within a founding population, or genetic drift: the loss of rare, non-selective alleles through random fluctuation of allele frequencies between generations, particularly in small populations (Nei *et al.*, 1975; Allendorf, 1986). Secondly by selective effects i.e. natural selection or sexual selection. Adaptation is the sorting of heritable phenotypic variation by natural selection. If an allele is associated with a fitness increase in the phenotype, then the allele will spread through the population over subsequent generations (Taylor, 1991). In sympatry, this may involve diversification through disruptive selection resulting in simultaneous alternative phenotypes often with morphological differences (these are often called polymorphisms or alternative morphotypes). Selection acts in different directions, where those individuals that are specialised have an advantage over intermediate phenotypes (Rueffler *et al.,* 2006). This might involve specialisations for two or more environments or resources that produce a fitness benefit in one environment, but a fitness disadvantage in others (Schluter, 1995; Dewitt *et al.,* 2000). Heritable variation can also be sorted by sexual selection (Iwasa & Pomiankowski, 1995; Irwin & Price, 1999). This may occur alongside natural selection, for instance through assortive mate choice i.e. with females choosing males more like themselves (Lande & Kirkpatrick, 1988; Rundle & Schluter, 1998; Higashi *et al.,* 1999). How this might emerge has been demonstrated by Garduño-Paz (2009) in experiments involving three-spined sticklebacks (*Gasterosteus aculeatus*).

However, phenotypic variation between and within sites can also be due to phenotypic plasticity, the different expression of the same genotype, usually as a response to environmental cues (West-Eberhard, 1989). This allows a fast response to a new environment or change in environmental conditions and the exploitation of novel resources. This also allows simultaneous alternative morphotypes to be maintained without reduction of gene flow between phenotypes, as well as through disruptive selection. In theory, phenotypic plasticity would seem to preclude the opportunity for speciation and genetic variation since individuals would always be near a fitness optima. However, moderate plasticity can actually facilitate genetic change (West-Eberhard, 1989; Agrawal, 2001; Price *et al.,* 2003).

Firstly, phenotypic plasticity can confer a significant advantage. In novel or heterogeneous environments, phenotypic plasticity expands the ecological range of a species, and as such

plasticity to varying extents is extremely common. This allows taxa to invade and prosper in new environments, where other processes may result in diversification from the donor population over time (Agrawal, 2001).

Secondly, the magnitude of plastic response may move in different directions in different environments. While conferring advantages under certain conditions, phenotypic plasticity can result in costs (Agrawal, 2001), for instance reduced growth (Van Buskirk & Relyea, 1998). There may also be disadvantages to plasticity in certain circumstances, for instance directly where there are ontogenetic changes in habitat or resource use, since a plastic response in an early life stage may have an effect on a later life stage (e.g. Orizaola & Laurila, 2009). In an extremely variable or unpredictable environment a plastic response may be disadvantageous over a longer term due to a time lag (DeWitt *et al.,* 1998), or possibly over generations if there is a maternal effect.

Finally, the direction and degree of a plastic response, the reaction norm, to environmental factors is genetically variable, thus genetically variable individuals will not show exactly the same plastic response to the same environment (West-Eberhard, 1989). Plasticity is therefore itself a trait that can be selected for and the range of plastic responses may change due to selection, in this way selection can favour the accumulation of functionally useful (and potentially novel) plastic responses (West-Eberhard, 2005; Parsons & Robinson, 2006). An environment in which one or more distinct plastic responses are strongly favoured can result in the genetic assimilation or fixing of phenotypes in a population so that it is expressed even when the original environmental stimulus is removed (Waddington, 1953; Adams & Huntingford, 2004; Pigliucci *et al.,* 2006). Thus plasticity can play a role in increasing differences between and within populations and thus potentially in speciation.

1.3 ECOLOGICAL CAUSES OF PHENOTYPIC VARIATION

There are a range of factors that can produce variation between and within sites either through selection or plasticity. The presence (or absence) of other species can have an effect on the phenotype of an organism. There are several examples of predator-mediated phenotype differences, for instance defensive and escape-response morphology in fish (Doucette *et al.,* 2004; Langerhans *et al.,* 2004), shape and thickness of gastropod shells (DeWitt *et al.,* 2000; Trussell & Smith, 2000), and timing of hatching as a response to egg predation (Warkentin, 1995; Jones *et al.,* 2003; Kusch & Chivers, 2004). Ecological character displacement can occur in the presence of a competitor species (Crowder, 1984;

Pfennig *et al.,* 2007). Character release can occur when competing species are absent; due to availability of resource opportunities and intra-specific competition this may result in simultaneous morphotypes (Robinson & Wilson, 1994; Robinson *et al.,* 2000).

Physical aspects of the habitat such as temperature (Booth, 2006), habitat type/complexity (Kostylev *et al.,* 1997; Goodman *et al.,* 2008), oxygen concentration (Langerhans *et al.,* 2007) and moisture (Johnson & Black, 1998; Brown & Shine, 2006) can also have a profound effect on the phenotype of an organism. There is also evidence of climate change influenced phenotypic changes in a variety of taxa (Post *et al.,* 1997; Galeotii *et al.,* 2009; Wolf *et al.,* 2009).

The exact factors influencing a phenotype can be difficult to unravel, even closely related taxa may respond differently to the same environment. Pakkasmaa & Piironen (2000) found phenotypically plastic differences in body height and fin size between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) juveniles raised in fast or slow water flow. While each responded plastically to the different habitats, the responses between the species were not the same with salmon juveniles becoming more robust and trout juveniles becoming more streamlined in fast flowing water. Moreover, different factors may have a similar effect on the phenotype. In fish, a deeper body shape is indicative of increased manoeuvrability and has been noted in species and alternative phenotypes that inhabit complex habitats (Webb, 1984), a deeper body can be indicative of feeding on benthic prey (Andersson *et al.,* 2006), and can also be a response to the presence of gape-limited predators (Jastrebski & Robinson, 2004; Domenici *et al.,* 2008).

However, when a certain set of characters is associated consistently with a specific habitat or feeding mode over many distinct taxa, this is usually indicative of a shared response to that environment, rather than any kind of evolutionary constraint. One of the most well-known of these are the differences are alternative feeding ecology between freshwater fish specialising in exploiting limnetic or benthic resources (Smith & Skulason, 1996; Robinson & Parsons, 2002; Parsons & Robinson, 2006). These differences are associated with trophic specialisations in morphology that have functional significance for prey detection, capture and handling. This can result in simultaneous limnetic/benthic phenotypes in one population (Smith & Skulason, 1996). Since, aquatic environments can be divided vertically and horizontally, some populations can have several simultaneous morphotypes which partition the available resources and specialise accordingly. Some

European whitefish (*Coregonus lavaretus*) populations can support up to five sympatric morphs (Svärdson 1979; Bergstrand, 1982).

Limnetic fish of virtually all taxa converge on a fusiform body shape that is hydrodynamically efficient (Webb, 1984). These tend to be plankton feeders which usually have a large number of closely spaced gill rakers (Lindsey, 1981; Lavin & McPhail, 1985, 1986; Schluter & McPhail, 1993). The mouth is orientated terminally in pelagic feeders, while in benthic feeders mouths are orientated downwards (Lindsey, 1981; Keast & Webb, 1966). Fish that exploit hard-bodied benthic prey have robust heads with increased musculature (Werner & Hall, 1979; Wainwright, 1991). These patterns are often (but not only e.g. Ruzzante *et al.,* 2008) seen in variable fish species that inhabit in northern post-glacial lakes. Typical highly variable postglacial fish species include three-spined sticklebacks (e.g. Baker *et al.*, 2005) and salmonids such as brown trout (e.g. Ferguson, 2004), Arctic charr (*Salvelinus alpinus*) (e.g. Jonsson & Jonsson, 2001) and European whitefish (e.g. Amundsen, 1988; Naesje *et al.,* 2004; Ostbye *et al.,* 2005a; Kahilainen & Ostbye, 2006). This phenotypic variation often has a plastic element, but can also be associated with genetic differentiation between simultaneous alternative morphotypes.

1.4 THE MANAGEMENT AND CONSERVATION OF PHENOTYPICALLY VARIABLE SPECIES: POSTGLACIAL FISHES

The phenotypic divergence between and within populations of post glacial fish species have often developed in relatively short time scales as indicated by relatively low levels of genetic divergence among species (Schluter, 1996; Bernatchez & Wilson, 1998). These inhabit areas influenced by climatic changes associated with the Pleistocene, a time of repeated glacial episodes which created variable conditions favouring rapid phenotypic divergence among northern postglacial fishes (Bernatchez & Wilson, 1998; Hewitt, 2004). This has led to a confused taxonomy in these and other phenotypically variable fish species.

Alternative morphotypes can occur with and without genetic divergence. There can, for example, be a continuum of variation in allopatry and sympatry from almost no-differentiation, to phenotypic polymorphisms that may be intermediate stages in speciation, to almost total reproductive isolation (Smith & Skulison, 1996; Schluter, 2001). This undelimited continuum can make identifying species for conservation or management purposes particularly unclear in plastic taxa. The conservation of simultaneous alternative phenotypes can be a particular problem, since their taxonomic status is usually unclear

(Taylor, 1999). Increasingly however, divergent populations are being recognised as appropriate for conservation, regardless of their taxonomic status. Sub-species, while in theory being a systematic unit, are controversial due to the finding that some sub-species are not evolutionary distinct and the historical inaccuracy with which this term is often associated (Lidicker, 1962; Zink, 2004). The evolutionary significant unit (ESU) is a commonly used infra-species unit for conservation (e.g. Legge *et al*. 1996; Stockwell *et al.*, 1998; Holycross & Douglas, 2007). The ESU was proposed by Ryder (1986) as a population "*possessing genetic attributes significant for present and future generations*". This purely genetic definition has been modified elsewhere to also include discrete phenotypic characters and restricted gene flow (see DeGuia & Saitoh, 2007 for review of its use).

Hundreds of years of research on classifying the variation within some of these groups has resulted in taxonomic confusion, typified by the 'charr problem' (Nordeng, 1983; Behnke, 1989; Nyman, 1989) and the 'coregonid problem' (Svärdson, 1949, 1950; Stott & Todd, 2007). At one point at least 40 different 'species' of three-spined sticklebacks were identified (Bell & Foster, 1994), while over 200 intra-specific forms of *C. lavaretus* have been formally described in Europe (Reshetnikov, 2004).

Coregonus species show phenotypic and genotypic variation between populations and between genetic sub-populations and simultaneous alternative morphotypes within populations due to a variety of processes (Bernatchez *et al.,* 1999). In coregonids similar patterns in morphology are often seen between and within sites. For example, gill raker number is often used to differentiate between sympatric phenotypes (Ilmast & Sterligova, 2002; Kahilainen *et al.,* 2003). These have a high hereditary component and so have also been used as a character to identify species (Svärdson, 1957). However, coregonids display homoplasy in a variety of traits, including gill raker number, associated with repeated divergence to exploit similar resources usually found in postglacial lakes and into alternative phenotypes utilising distinct resources in a single site. As with many postglacial fishes this often revolves around the littoral/benthic polymorphism (Ostbye *et al.,* 2005b). Gill rakers also have a plastic component (Lindsey, 1981). Plastic responses are well known in *Coregonus* species, for instance in response to commercial and experimental translocations (Svärdson, 1950, 1979). The infra-species differences between some populations of species such as the European whitefish that are commercially exploited have not been appreciated until recently. This had led in some cases to management

practices that have involved mixing various populations to the detriment of conservation and study of adaptive divergence and species formation (Douglas & Brunner, 2002).

Coregonus spp. in Europe are listed under Appendix iii of the Bern Convention and are considered to be either endangered or vulnerable due to threats such as eutrophication, acidification, over fishing or species introductions (Lelek, 1987; Winfield, 1992; Winfield *et al.,* 1996, 1998; Winfield & Durie, 2004). The introduction, by accident or design, of alien species into a site is one of the principal threats to fish conservation (Cambrey, 2003; Copp *et al.,* 2005; Casal, 2006). Once a new species is established it may be difficult or impossible to remove. It can have detrimental effects on other species directly though competition (Salo *et al.,* 2007) or predation (Letnic *et al.,* 2008), or indirectly through the associated introduction of disease (Pinder *et al.,* 2005) or modification of the environment (Matsuzaki *et al.,* 2009). However, invasive species can also offer insights into evolutionary and plastic responses in both invading species and native species (Carroll, 2007; Prentis *et al.,* 2008) to novel environments and changing species interactions. The effect of stressors and conservation actions may have important implications for the future of coregonids. Responses to stresses, such as the addition of alien species, eutrophication, or long term climate change might involve change in the phenotype and/or genotype, or cause the loss of a stable polymorphism and thus effect *in situ* management. Furthermore, *ex situ* conservation actions such as the creation of refuge populations involve deliberate translocation of species that have the potential for fast phenotypic change to a novel environment.

1.5 *COREGONUS SPP*. IN THE UK

There are four species of *Coregonus spp*. (whitefish) currently recognised as recently occurring in the Britain and Ireland. The houting *Coregonus oxyrinchus* L., Arctic cisco (pollan) *Coregonus autumnalis* Thompson, vendace *Coregonus albula* L. and European whitefish (powan, schelly or gwyniad) *C. lavaretus* L. These populations of *Coregonus* are thought to have originated from anadromous ancestors that migrated into freshwaters after the last glaciation, some 10,000 years ago (Maitland, 1970; Wheeler, 1977). Most (but not all) of these stocks lost their migratory habits and are now found only in fresh water lakes.

The houting (*C. oxyrinchus*) was the only anadromous whitefish in Britain where it was found only in the coastal areas of the southwest of England (Maitland, 2004a). It is now believed to be locally extinct due to pollution in the rivers in which houting spawned (Maitland & Lyle, 1991a; Freyhof & Schoeter, 2005). Although this is considered to be a

separate species, recent genetic work by Hansen *et al.*, (2008), has suggested that European populations of houting may in fact be conspecific with *C. lavaretus*.

Pollan (*C. autumnalis*) is the only coregonid species found in Ireland, occuring in Loughs Neagh, Erne, Fee and Derg. Historically, pollan were regarded as conspecific with one of the other UK coregonids. (Svärdson, 1957; Dottrens, 1959; Maitland, 1972a; Wheeler, 1969; 1977, but see Yarrell, 1836), while Tate Regan (1906; 1908) considered there to be more than one species of pollan in Ireland. Genetic analyses (e.g. Ferguson, 1974, Ferguson *et al.*, 1978) have demonstrated this not to be the case. These *C. autumnalis* populations are far to the south of the usual range of Arctic cisco, and show atypical life histories (Harrod *et al.,* 2001) though there is some evidence of the anadromous tendencies usually found in this species (Welch, 1927; Twomey, 1956; Wilson, 1984). There is a long history of exploitation of these populations, particularly those of Lough Neagh (Hamilton, 1843; Thompson, 1856), which is also home to the only other known population of resident river lamprey (*Lampetra fluviatilis*) outside Loch Lomond (Goodwin *et al.,* 2006). There are a number of threats to these populations, in particular the pollan loughs are now dominated by introduced cyprinids and percids (Quigley & Flannery 1996; Harrod *et al.*, 2001), as well as affected by eutrophication. There is electrophoretic evidence of the presence in Lough Neagh of several sub-populations of pollan which are to some extent reproductively isolated (Ferguson, 1975 and current work by Bradley *et al.*, Pers. Comm.), though this has not been associated with alternative phenotypes.

Vendace (*C. albula*) is the rarest freshwater fish in Britain. Only four populations were known to have been located in Britain. Two of these (Castle Loch and Mill Loch) were located in Lochmaben, Scotland, and have been extinct since the 1960s. Here it was a particularly celebrated fish, where some traditions and curious opinions existed regarding it (Jardine, 1830; Yarrell, 1836). Local legends have variously asserted that vendace was introduced to Lochmaben by Mary Queen of Scots or by Robert the Bruce (Jardine, 1830; Richardson, 1836; Yarrell 1836; Day 1884). Eutrophication and fish introductions have been suggested as the principal reasons for their extinction at these sites (Maitland, 1970; Maitland, 1966; Winfield *et al.*, 1996). Until recently two English populations were found in Bassenthwaite and Derwent Water in the English Lake District. Tate Regan (1906) believed these Scottish and English populations to be two different species. Whilst the Derwent Water population of vendace is still extant, the Bassenthwaite population has been extirpated, due to eutrophication and fish introductions (Mubamba, 1989; Winfield *et al.,* 2004), which are becoming a problem in the Derwent Water population. Conservation

action, however, has resulted in several refuge populations having been established from both the Bassenthwaite and Derwent Water populations (Maitland & Lyle, 1990; Maitland *et al.*, 2003). Overall the British *C. albula* populations are less well-studied than the *C. autumnalis* and *C. lavaretus* populations (but see Tate Regan, 1906, 1908, 1911; Maitland 1966, 1970, 1979; and Ferguson, 1974).

Table 1.1 Authors of different species designations for the Scottish, English and Welsh populations of *C. lavaretus*.

Coregonus	British whitefish	Lomond	Eck	Brotherswater	Haweswater	Red Tarn	Ullswater	Tegid
acronius	Schindler, 1957 (from Maitland, 1970)							
cepedii		Valenciennes, 1848						
clupeoides		LaCepede, 1803, Gunther, 1866; Day, 1884; Tate, 1908; Kottelat & Freyhof, 2007	Tate, 1911; Kottelat & Freyhof, 2007		Guther, 1866; Day, 1884; Tate, 1908	Day, 1884	Guther, 1866; Day, 1884; Tate, 1908	Guther, 1866; Day, 1884; Tate, 1908
fera	Yarrell, 1836							
lacepedei		Parnell, 1838						
lavaretus	Linnaeus, 1757; Pennant, 1776; Turton, 1807; Fleming, 1828;,Jenyns, 1835; Steinmann, 1950	Jardine, 1830; Svardson, 1957; Gasowka, 1965; Maitland, 2004a	Maitland, 2004a	Maitland, 2004a	Maitland, 2004a	Maitland, 2004a	Jardine, 1830; Maitland, 2004a	Gasowka, 1965; Maitland, 2004a
macrophthalmus					Drottrens, 1959			Drottrens, 1959
microcephalus		Parnell, 1838						
oxyrhynchus					Svardson, 1957		Svardson, 1957	Svardson, 1957
pennantii								Valenciennes, 1848; Tate, 1911; Kottelat & Freyhof, 2007
stigmaticus				Kottelat & Freyhof, 2007	Tate, 1911; Kottelat & Freyhof, 2007	Tate, 1911; Kottelat & Freyhof, 2007	Tate, 1911; Kottelat & Freyhof, 2007	
wartmanni	Yarrell, 1836;	Drottrens, 1959					Drottrens, 1959	

The seven European whitefish (*C. lavaretus*) populations are variously known as powan (Scotland), schelly (England) and gwyniad (Wales). The taxonomy of these populations has been the subject of considerable debate and they have been designated a great number of different species names by various authorities (Table 1.). Powan are found in Loch Lomond and Loch Eck, in west-central Scotland. Schelly are found in Brotherswater, Haweswater, Red Tarn and Ullswater in the Lake District. Gwyniad are found in Llyn Tegid in North Wales. The location and physical characteristics of these study sites are described in Appendix 1. *C. lavaretus* have also been reported, but never confirmed, in Loch Morar (Murray & Pullar, 1910) in Scotland, Conningham Mere, Pemble Mere (Day, 1884) and Loweswater (Ellison, 1966a) in England. However, since there has been no confirmation of the existence of coregonids at these sites they will not be considered further in this thesis.

Loch Lomond powan have been commented on extensively and are the best studied population of *C. lavaretus* in the UK (e.g. Monipenny, 1612; Sibbald, 1694; Sinclair, 1799; Lamond, 1931; Gervers, 1954; Slack, 1955, 1957; Maitland, 1969; Roberts *et al.*, 1970; Fuller *et al.*, 1974; Brown & Scott, 1987; Brown *et al.*, 1991; Pomeroy, 1994; Dorucu, 2000). However, little is known about the early life history of powan as 0+ fish are difficult to catch in the wild, the only large catch of juvenile powan in Loch Lomond (> 200) was made in July 1969 (Slack *et al.*, 1957; Brown & Scott, 1994).

Loch Lomond powan feed intensively on zooplankton from May to September showing inter- and intra-specific size-selectivity of zooplankton prey (Pomeroy, 1991, 1994). However, in the winter feeding rate is reduced and they take small quantities of benthic prey. Diel migration relating to low light intensities occurs: powan migrate to the surface layers and inshore during the evening twilight and return to deeper water at dawn (O'Connell, 1984)

Loch Lomond has the widest variety of indigenous and introduced fish species found in any freshwater body in Scotland (Maitland, 1972b; Adams 1994; Etheridge & Adams, 2008). It is thought that an introduced species, the ruffe (*Gymnocephalus cernuus*), had a detrimental effect on powan through egg predation (Adams & Tippet, 1991; Maitland, 1995; Adams & Maitland, 1998). *C. lavaretus* broadcast spawn on shallow littoral areas or off-shore banks and thus provide no protection for their eggs unlike many salmonids that build nests or 'redds' (e.g. Sargent *et al.*, 1987). In Loch Lomond powan eggs spend between 60 and 70 degree days incubating on the spawning grounds before they hatch, during which there is a high mortality (Slack *et al.*, 1957; Brown *et al.*, 1991; Winfield *et al.*, 1996). Change in the growth patterns and life history of Loch Lomond powan might be expected if ruffe predation were altering numbers significantly, although this does not currently appear to be the case (Appendix 5).

Until recently, the total number of powan in Loch Lomond was believed to be numerous (Slack *et al.*, 1957; Brown & Scott, 1994); Wood (1947) describes 'great shoals of powan'. However, recent hydroacoustic monitoring at Loch Lomond has indicated (alongside Chapter 3 in this thesis) that there has been a decline in powan at this site (Winfield *et al.*, 2005, 2008a). In hydroacoustic surveys and associated gill netting in 2004 as part of site condition monitoring by Winfield *et al.* (2006a), population density of powan was calculated to be 6.3 fish ha^{-1} (95 % confidence intervals (CI): 3.5 fish ha^{-1} and 11.6 fish ha^{-1}), small individuals were calculated as making up 37 % of the population (CIs: 14 % and

60 %). These surveys were repeated in 2007 (Winfield *et al.*, 2008a), population density of powan was calculated to be 1.8 fish ha^{-1}, (CIs: 0.8 fish ha^{-1} and 4.4 fish ha^{-1}), small individuals were calculated as making up 51 % of the population (CIs: 29 % and 73 %). This can be compared to a netting survey in Loch Lomond by Bean (1996) which calculated a powan population density of 13.1 fish ha^{-1} (from Winfield *et al.*, 2006a). This indicates a low abundance of powan at this site with low numbers of small fish (0+/1+ years) suggestive of low recruitment (Winfield *et al.*, 2006a), although *C. lavaretus* is characterised by variable recruitment (Winfield *et al.*, 1994). According to monitoring protocols a population made up of 90 % small individuals is in favourable condition (Bean, 2003).

Due to concerns about the long-term status of powan in Loch Lomond following the introduction of ruffe, powan from the Loch Lomond population were translocated to form two refuge populations in Loch Sloy and Carron Valley Reservoir Powan between 1988 and 1991 (Maitland & Lyle, 1992; 1995). Relatively little is known about powan from the refuge sites. However, hydroacoustic surveys were undertaken at both refuge sites in 2007 as part of site condition monitoring by Winfield *et al*. (2008b). In Loch Sloy, the population density of powan was calculated to be 6.4 fish ha^{-1} (CIs: 2.6 fish ha^{-1} and 16.0 fish ha^{-1}), small individuals were calculated as making up 81 % of the population (CIs: 25 % and 137 %). Thus while there was low abundance at this site, there was evidence of recent recruitment. The only other species that appears to occur alongside powan at this site is brown trout. Carron Valley Reservoir is a trout fishery, a study on growth and feeding of brown trout by Deverill (2000) also resulted in information on the Carron Valley Reservoir powan. These powan have a faster growth rate that those from other sites and appear to feed on a mixture of planktonic and benthic prey (Deverill, 2000). In Carron Valley Reservoir, the population density of powan was calculated to be 17.2 fish ha^{-1} (CI: 5.6 fish ha^{-1} and 52.7 fish ha^{-1}), small individuals were calculated as making up 25 % of the population (CIs: -14 % and 64 %). Thus at this site there was low abundance at this site and little evidence of recent recruitment (Winfield *et al.*, 2008b)

Despite being a protected feature within a Site of Special Scientific Interest (SSSI), powan in Loch Eck have been less well studied than those from Loch Lomond. However, some studies (e.g. Pomeroy, 1991; Brown & Scott, 1994) have shown that Loch Eck fish differ from Loch Lomond powan in diet and life history parameters. In Loch Eck, powan feed on benthic material throughout the year as well as some zooplankton (Pomeroy, 1991). Loch Eck powan are shorter at a given age than Lomond fish, but the weight at a given length of

Loch Eck fish is consistently greater than Loch Lomond fish (Brown & Scott, 1994). Loch Eck fish also mature at a younger age; have a lighter parasite load (Dolezel & Crompton, 2000) and spawn slightly later in the year than Loch Lomond powan (Brown & Scott, 1994). Despite these differences, some studies have suggested there is no genetic difference between powan from Loch Lomond and Loch Eck (Hartley, 1995). In hydroacoustic surveys and associated gill netting in 2005 as part of site condition monitoring in Loch Eck by Winfield *et al*. (2006b), population density of powan was calculated to be 135.8 fish ha^{-1} (CIs: 60.4 fish ha^{-1} and 305.5 fish ha^{-1}). These surveys were repeated in 2007 (Winfield *et al.*, 2008a), population density of powan was calculated to be 203.9 fish ha^{-1}, (CIs: 137.4 fish ha^{-1} and 302.7 fish ha^{-1}), small individuals were calculated as making up 98 % of the population (CIs: 77 % and 118 %). This population therefore appears to be very health, with good recent recruitment.

The presence of schelly in Brotherswater was confirmed only relatively recently. Its presence at Brotherswater was indicated by Ellison (1966a), but it was not until 1992 that the fish community of this site was surveyed (Winfield *et al.*, 1993) and *C. lavaretus* were verified as being extant at this site. However, this survey also revealed that this population of schelly was dominated by old individuals suggesting poor recruitment at the time of survey. It also demonstrated low oxygen conditions at times in deep water and intimated that this might be a significant issue for their continued survival (Winfield *et al.*, 1993).

Schelly populations are monitored by the Environment Agency (EA), Centre of Ecology and Hydrology (CEH) and United Utilities in England. This is particularly true of Haweswater which is the most extensively monitored of the English *C. lavaretus* populations (i.e. Winfield *et al.*, 2006c). Information on the status of schelly in Haweswater reservoir is obtained from routine gill net and hydroacoustic surveys and augmented from data relating to the entrainment of fish in trash screens at water abstraction points. This has enabled the discovery of a decline in the Haweswater populations and modelling of the population in relation to the effect of reservoir operations (e.g. fluctuating water levels) and predation by a recently established colony of cormorants *Phalocrocorax carbo* (Winfield *et al.*, 1998, 2003, 2004). This is the only other population in Britain apart from Loch Eck, in which whitefish and Arctic charr exist in sympatry. A comparative study between the two populations found that Haweswater schelly were slower growing than Ullswater schelly (Bagenal, 1970). Haweswater schelly feed near the bottom of the loch, but also shoal on the surface at night and so may also feed on plankton (Swynnerton & Worthington, 1940; Dunn, 1954). Concern regarding the continued

survival of the schelly at this site has led to the establishment of two refuge populations using parental stock from Haweswater at Blea Water and Small Water respectively (Winfield *et al.*, 2002).

In Red Tarn, schelly were confirmed to be extant by Maitland *et al.* (1990) though there were various accounts of its existence at this site (Ellison, 1966a). Red Tarn schelly were found to feed on plankton and the growth of schelly from this population is much slower than that of any other population of British *C. lavaretus*. There have been suggestions that the populations of schelly in Red Tarn originated from human introductions (Macpherson, 1892), however, both Day (1884) and Maitland *et al.* (1990) considered this unlikely due to the remote nature and difficulty of accessing this site, in conjunction with the lack of value of this fish for anglers. Red Tarn schelly differ genetically from other schelly possibly due to a small founding population, or selection due to the high altitude of this lake (Beaumont *et al.*, 1995).

Ullswater schelly are mainly planktivorous, but also feed on benthos (Bagenal, 1966). They spawn in shallow areas and, around spawning time, are sometimes washed ashore in mass strandings down-wind of the spawning sites (Bagenal, 1966; Ellison, 1966b). Some Ullswater schelly display a supernumery pelvic fin (Bagenal, 1970). While there is no current fishery for schelly in any of the English lakes, according to Mubamba (1989), local fishermen claimed that the population in Ullswater was exploited as recently as the early 1920's.

Llyn Tegid holds the only Welsh population of *C. lavaretus* though, according to Day (1884), a gwyniad was recorded in one instance 10 km downstream of the lake, within the River Dee. It has been suggested that there is more than one whitefish type in Llyn Tegid (Dottrens, 1959, but see Gasowka, 1965; Haram, 1968). The parasitology of Llyn Tegid fish has been relatively well studied (e.g. Chubb, 1962, 1963), including that of gwyniad (Nicholas & Jones, 1959; Chubb, 1963; Daniels, 1982). Gwyniad are primarily bottom feeders, although there is some evidence that plankton forms an important part of the diet during the summer months (Haram, 1968). There may be an ontogenetic change in diet with larger fish switching to a plankton dominated diet (Dunn, 1954). Early echo sounding surveys by Haram (1968) revealed complex diurnal and seasonal changes in fish distribution within Llyn Tegid. More recent hydroacoustic surveys have attempted to establish the current status of gwyniad at this site as part of a wider programme of Site Condition Monitoring by country conservancy agencies (e.g. Winfield *et al.*, 2007a). In

general however, there has been very little recent ecological work on gwyniad, possibly due to restrictions on netting. However, attempts have been made to establish one refuge population at Llyn Arenig Fawr (Winfield *et al.,* 2008c).

In the UK all members of the genus *Coregonus* are Biodiversity Action Plan (UKBAP) priority species and are protected under Schedule 5 of the Wildlife and Countryside Act 1981. Alien or locally non-native fish introductions into most British whitefish sites have already occurred (Winfield & Durie, 2004; Winfield *et al.,* 2007b; Etheridge & Adams, 2008). The effect of these introductions, and the introduction of non-native plants (such as New Zealand pygmyweed *Crassula helmsii*) on whitefish populations are hard to quantify but for the most part are considered to be negative (e.g. Adams & Tippet, 1991). Eutrophication and the associated siltation of spawning grounds (Ventling-Schwank & Livingstone, 1994) has been a problem in some sites, resulting (alongside species introductions) in the extinction of vendace in Scotland (Maitland, 1966; Winfield *et al.,* 1996). Whitefish are cold adapted (Slack *et al.,* 1957; Bagenal, 1966, 1970) and climate change may be a particular problem in future years. Increased temperatures have already been noted at *Coregonus spp*. sites where this is being monitored (Krokowski, 2007) along with other climate change indicators (Salama *et al.,* 2007). Unless a site has a thermal refuge (i.e. depth, high altitude), whitefish may be adversely affected by warmer temperatures (Graham & Harrod, 2009). Warmer temperatures may also facilitate further invasions by non-native species or alter the scale of competitive or predatory interaction within formerly stable ecosystems. How these phenotypically plastic fish might respond to these pressures remains to be seen. Thus the investigation into aspects of the conservation of British *C. lavaretus* at this time is opportune and studies into these populations may also inform conservation and management of similar species.

1.6 THESIS AIMS

The main focus of this study is to investigate the conservation status of *C. lavaretus* in the UK. In addition, this study will elucidate the effects of the variation commonly found within and between populations of this fish on management options for UK *C. lavaretus* using the relatively well-studied Loch Lomond population as a case study. This thesis aims to deliver definite management proposals based on the findings of the following six studies:

1. A recent review of the species status of European freshwater fishes has been completed by Kottelat & Freyhof (2007). Chapter 2 aims to investigate whether

resulting revisions in the species status of *C. lavaretus* in the UK can be justified at this time.

2. Variation between *C. lavaretus* populations would effect whether populations can be treated as one widespread management unit. Differences between the native Scottish populations are investigated in Chapter 3 with a view to producing management advice on their status as ESUs.

3. In the UK, the creation of refuge populations of *Coregonus spp*. is becoming more common. The implications of translocation of a phenotypically plastic species into a novel environment for conservation purposes are investigated in Chapter 4 using donor and refuge populations of *C. lavaretus* in Scotland.

4. Within population variation in simultaneous phenotypes or genetic sub-populations of *C. lavaretus* can have important implications for conservation action *in situ* and *ex situ*. Since a historical report by Parnell (1838) identified two morphologically distinct powan 'species' in Loch Lomond, sub-structuring in this population is investigated in Chapter 5.

5. In the UK, invasive ruffe have been introduced to several sites containing *C. lavaretus*. In Loch Lomond these fish are known to feed on *C. lavaretus* eggs. In Chapter 6 the protective ability of substrates found on *C. lavaretus* spawning grounds against ruffe foraging is tested experimentally.

6. Another phenotypically plastic species found in Loch Lomond is brown trout. These typically follow discrete life histories of residence or anadromy that result in alternative phenotypes. In Loch Lomond it was observed that many brown trout appear to have an intermediate phenotype not obviously consistent with one or other life-choice. Thus phenotypic plasticity within a population is investigated in Chapter 7 using stable isotope analysis.

Appendices contain general information about the study sites and populations that were not substantial enough to form chapters. Nevertheless these hold information that is illuminating in a discussion of the six main studies. Appendix 1 gives a general description of the study sites. Appendix 2 describes the catch composition of gill netting at the two native Scottish populations of *C. lavaretus*, Loch Lomond and Loch Eck. Appendix 3 gives

an account of the muscle tissue stable isotope analysis values of Loch Lomond fish. Appendix 4 describes the muscle tissue stable isotope analysis values of native and refuge populations of *C. lavaretus* from Scotland. Appendix 5 describes a static life table for Loch Lomond *C. lavaretus*.

The results of the preceding chapters and additional results presented in appendices are discussed in chapter 8. This also contains suggestions for management and conservation of *C. lavaretus* in the UK, and additional work that should be completed in light of the results presented in this thesis.

Chapter 2 AN EVALUATION OF THE EVIDENCE FOR SPECIATION OF UK WHITEFISH.

2.1 ABSTRACT

Whitefish are amongst the most threatened vertebrates in the UK and as such require effective conservation and legal protection. There are serious potential implications of species name changes affecting different aspects of the management, conservation and protection of whitefish populations. The systematics of whitefish are relatively well studied, but there is little agreement at species level. Kottelat & Freyhof (2007) in *The Handbook of European Freshwater Fishes* claim to have cleared up much of the uncertainty. This designates UK *Coregonus lavaretus* populations into three putative species by geography *C. clupeoides* (Scotland), *C. stigmaticus* (England) and *C. pennantii* (Wales). The keys used to split UK populations are investigated. Moreover, the success of splitting populations using the characters provided in the key by multivariate techniques is investigated. The success rate of the key is very low (19%) and the evidence for the splitting of these populations into three species is conflicting. This is discussed in relation to previous work on the systematics of UK whitefish. Due to the lack of evidence for these putative species, it is suggested that further work is needed before they can become accepted. Moreover, until evidence is provided otherwise, these should remain as individually managed populations of *C lavaretus* which are legally protected within the UK.

2.2 INTRODUCTION

The definition of which species and populations do and do not comprise a species is an important biological concept with significant consequences. Definition of species allows diversity to be catalogued and managed. The species definition is particularly important as a political and conservation concept, as it is generally species that are protected by law, discussed in the media or recognised by the general public. It is also essential for information exchange. How to define a species, however, is not completely clear. There are at least 22 different definitions of what constitutes a species in current use (Mayden, 1997; Adams & Maitland, 2007). This is a particularly significant issue for groups that can show great variation between and within sites. Several freshwater fish living in postglacial lakes in particular show significant variation, these include three-spined sticklebacks (*Gasterosteus aculeatus*) (Campbell, 1985; Jones *et al.,* 2006), Arctic charr (*Salvelinus alpinus*) (Hartley *et al.,* 1992; Adams *et al.,* 2007) and whitefish (*Coregonus spp*.) (Bergstrand, 1982; Amundsen, 1988; Kahilainen *et al*., 2003, 2004). However, the detailed

discussion of species concepts in comparison to others is out-with the remit of the current work and is discussed in detail elsewhere (Turner, 1999; Coyne & Orr, 2004).

Coregonine fishes are found throughout the cooler regions of the northern hemisphere, and are renowned for the level of phenotypic variation displayed both between and within populations, resulting in long and marked debates regarding the allocation and definition of species (Berg, 1962; Wheeler, 1969; McPhail & Lindsey, 1970). Phenotypic plasticity (Lindsey, 1981; Svärdson, 1949), different possible evolutionary scenarios (i.e. allopatric vs. sympatric speciation) (Bernatchez & Dodson, 1990; Douglas *et al.*, 1999, 2005), and introgression (Svärdson, 1957), have all been identified as being responsible for the existence of different coregonine forms (or morphs, sub-species) and complicates the use of morphological and anatomical characters in their taxonomy. Traditionally, whitefish have been segregated into forms by counts of meristic characteristics, such as gill rakers, which have a high heritable component (Svärdson, 1950; 1951). However in fish, some meristic characters can be affected by interactions with other species (Lindsey, 1981), or contrasting environmental conditions (Svärdson, 1951; Lindsey, 1962; Swain & Lindsey, 1986). The radiation of extant coregonine species has occurred comparatively recently; the relatively low levels of genetic variation contrasts with the high level of morphological differentiation observed among coregonines (Bernatchez *et al.*, 1991). It has therefore been suggested that genetic analyses are better at revealing phylogenetic patterns, while morphological patterns are better indicators of adaptive processes in these species (Lindsey, 1981; Bernatchez *et al.*, 1991).

In recent times three species of coregonids have been recognised as occurring naturally in the UK and Ireland; the Irish pollan *Coregonus autumnalis* (Pallas), the vendace *Coregonus albula* L. and the European whitefish *Coregonus lavaretus* L. locally referred to as powan (Scotland), schelly (England) or gwyniad (Wales) respectively. The British populations of *Coregonus* are thought to have originated from anadromous ancestors that migrated from a refuge after the last glaciation, some 10,000 years ago, extant populations are now landlocked and are purely fresh water in occurrence (Maitland, 1970). The houting (*Coregonus oxyrinchus*) was the only anadromous whitefish in Britain it was rarely recorded but is considered to be absent from the UK fauna (Maitland & Lyle, 1991a; Freyhof & Schoeter, 2005).

In Britain, *C. lavaretus* and *C. albula* are afforded legal protection due their rarity. Two populations of powan occur in Scotland in Loch Lomond and Loch Eck, and two refuge

populations have been established using parental stock from Loch Lomond, in Loch Sloy and Carron Valley Reservoir (Maitland & Lyle, 1990). Four populations of schelly occur in the Lake District, England, in Brotherswater, Haweswater, Red Tarn, and Ullswater. Two refuge populations have been established through the transfer of parental stock from Haweswater to Blea Water and Small Water (Winfield *et al.*, 2002). One population of gwyniad occurs in Wales in Llyn Tegid and one refuge population has been established in Llyn Arenig Fawr (Winfield *et al.*, 2008c). Vendace have a more limited range than *C. lavaretus*, From the four known populations, Castle Loch and Mill Loch (Scotland), and Bassenthwaite Lake and Derwent Water (England), only Derwent Water remains extant (Winfield *et al.*, 2004). One refuge population has been successfully established, at Loch Skeen, using parental stock from Bassenthwaite Lake. The success of two other translocations using parental stock from Derwent Water to Daer Reservoir, and to Sprinkling Tarn, have yet to be evaluated (Maitland *et al*, 2003). The conservation interest in these taxa makes the understanding of species level diversity in Britain particularly crucial.

The generally accepted view of coregonid taxonomy that dominates the current thinking of many fish biologists has been challenged by Kottelat & Freyhof (2007), who claim to have clarified much of the uncertainty in the systematics of the Coregonidae. Kottelat & Freyhof (2007) recognise 59 separate *Coregonus* species across Europe and suggest that there are many more likely to be accepted in future years. Within the UK, the seven native populations of *C. lavaretus* (*C. lavaretus* will be used as a general name describing all powan, schelly and gwyniad populations) have been identified as different endemic species by Kottelat & Freyhof (2007). According to these authors, both Scottish populations of *C. lavaretus* (powan) are endemic putative *C. clupeoides* (LaCepède), all four English populations of *C. lavaretus* (schelly) are endemic putative *C. stigmaticus* (Tate Regan), and the single Welsh population of *C. lavaretus* (gwyniad) is endemic putative *C. pennantii* (Valenciennes). All UK vendace are considered to be endemic putative *C. vandesius* (Richardson).

This paper focuses on coregonids in the U.K. that in the current literature are considered to be populations of *C. lavaretus* (i.e. seven native populations and two refuge populations). The aim is to critically examine Kottelat & Freyhof (2007) descriptions of this taxon in the UK. The historical division of these geographically separated populations into different species is described, to examine how these differ from this most recent examination of coregonid systematics, alongside more recent morphological and genetic data on these

populations. In order to test the accuracy of these new designations, the keys provided by Kottelat & Freyhof (2007) for the discrimination of these putative species are tested. The null hypothesis was that there was no significant difference between the identified putative and actual species. All definitions used henceforth are defined in Table 2.1.

Table 2.1 Glossary explaining name and character terms used in this chapter.

	Definition
Names	
Putative *spp.*	Proposed species name by Kottelat & Frehoff (2007).
Putative *C. clupeoides*	Whitefish from Loch Lomond, Loch Eck, Loch Sloy and Carron Valley Reservoir in Scotland
Putative *C. stigmaticus*	Whitefish from Brotherswater, Haweswater, Red Tarn and Ullswater in England
Putative *C. pennantii*	Whitefish from Llyn Tegid in Wales
C. clupeoides	Outcome of UK Coregonids Key in Kottelat & Freyhof (2007) The Handbook of European Freshwater Fishes.
C. stigmaticus	
C. pennantii	
British whitefish	Populations of putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*
Characters	
EDxSL	Eye diameter times in snout length: the number of times the eye diameter can fit in the snout length
GR	Number of gill rakers on first gill arch
Spots	Presence or absence of 'small blackish spots'
IODxHL	Interorbital distance times in head length: the number of times the interorbital distance can fit in the head length.
ScLP	Number of scale rows between the lateral line and the pelvic fin base
ScC	Number of circumpeduncular scale rows
AFR	Number of branched fin rays on the anal fin

2.3 METHODS

2.3.1 Sites

Four study sites are situated in west central Scotland. Loch Lomond and Loch Eck (natural populations), Loch Sloy and Carron Valley Reservoir (refuge populations). Four study sites are located in North England in the Lake District. Brotherswater, Haweswater which is a reservoir, Red Tarn in the Ullswater catchment, and Ullswater. One study site is located in Wales at Llyn Tegid (for additional details see Appendix 1 & Table 2.2.).

2.3.2 Fish Collection

British coregonids were collected from various populations of *C. lavaretus* (Table. 2.2.) Multi-panel Nordic-pattern benthic gill nets, which comprise 12 panels, ranging from 5 to

55 mm, knot-to-knot mesh, were set in each of the sites except Haweswater where fish were caught in water abstraction. Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986).

Table 2.2 Study site and fish capture information. SA = surface area, MD = maximum depth, ASL = height above sea level. GN = number of gill nets set, N = number of *C. lavaretus* captured, Abst = abstraction

	Site data						Netting data		
	Area	**Lat. Long.**	**SA (km2)**	**MD (m)**	**ASL (m)**	**Population Status**	**Dates**	**GN**	**N**
Loch Lomond	Scotland	56°05'N, 4°36'W	71.0	190	8	natural	09 Nov 05 – 24 Jan 06 15 May 08 – 23 Jul 08	75 13	118 10
Loch Eck	Scotland	56°06'N, 4°59'W	4.6	42	9	natural	09 Jan 06 29 Jul 08	6 3	223 40
Loch Sloy	Scotland	56°16'N, 4°47'W	1.0	Ca.40	287	refuge	21 Dec 05 – 28 Dec 05 28 Jul 08	7 4	76 40
Carron Valley Reservoir	Scotland	56°02'N, 4°06'W	3.0	Ca.10	223	refuge	03 Jan 06 – 05 Jan 06 31 Jul 08	10 2	58 9
Brotherswater	England	54°30'N, 2°55'W	0.19	16	173	natural	04 Jul 08	2	19
Haweswater	England	54°03'N, 2°48'W	3.9	57	246	natural	Winter 07/08	Abst	11
Red Tarn	England	54°31'N, 3°31'W	0.098	25	718	natural	15 Aug 08 & 05 Sept 08	5	22
Ullswater	England	54°34'N, 2°54'W	8.9	63	145	natural	04 Jul 08 – 12 Sept 08	18	29
Llyn Tegid	Wales	52°54'N, 3°37'W	4.14	42	170	natural	15 Dec 08	3	46

Putative *C. clupeoides* were sampled from Loch Lomond, Loch Eck, Loch Sloy and Carron Valley Reservoir, over the winter of 2005/06. During this period, a total of 475 putative *C. clupeoides* were caught, and in the summer of 2008 during this period a total of 99 putative *C. clupeoides* were caught. Putative *C. stigmaticus* were sampled from Brotherswater, Red Tarn and Ullswater in the summer of 2008, Haweswater fish were entrapped in reservoir operations over winter 2007/08. During this period a total of 81 putative *C. stigmaticus* were caught. Putative *C. pennantii* were sampled from Llyn Tegid in winter 2008 and during this period a total of 46 putative *C. pennantii* were caught. All fish were processed or frozen within four hours of capture.

2.3.3 Laboratory examination

In the laboratory, fish were thawed if necessary and fork length (L_f) (to 1 mm) and weight (W) (to 0.01 g) were measured. Measurements (to 0.1 mm) and meristic counts that are featured in the UK Coregonid key were taken from the fish (Table 2.3.). The first gill on the left branchial arch was removed and stored in 70 % ethanol; the gill-rakers were then counted (Kahilainen & Ostbye, 2006). The adipose fin was removed and stored in 100% ethanol for genetic analysis (reported on by Thompson *et al.*, 2008).

Table 2.3 Characters defining UK whitefish taxa, extracted from Kottelat & Freyhof (2007).

Key dichotomy	*C. pollan*	*C. oxyrinchus*	*C. stigmaticus*	*C. clupeoides*	*C. pennantii*
1	terminal mouth	sub-terminal mouth			
2		Anadromous Snout about 2 times eye diameter 36-44 gill rakers	Lacustrine Snout <2 times eye diameter 29-41 gill rakers		
3			Spots present Head length about 3.0-3.3 times interorbital distance	Spots absent Head length about 3.3-3.7 times interorbital distance	
4				7.5-8.5 scale rows between lateral line and pelvic fin base 20-21 circum-peduncular scale rows 9-11.5 anal fin rays	8-10 scale rows between lateral line and pelvic fin base 22-24 circum-peduncular scale rows 11-13.5 anal fin rays

2.3.4 Museum Specimens

Museum specimens were also examined. These provided additional specimens for examination, as only limited netting for fresh specimens was possible. Secondly, the fish caught in this study were usually required for several purposes which would be compromised by preserving the intact fish as recommended by Kottelat & Freyhof (2007). Fish examined in the Natural History Museum, London were preserved in formalin and stored in alcohol. In total 56 specimens were examined: putative *C. clupeoides* from Loch Lomond (n = 11) and Loch Eck (n = 3), specimens of putative *C. stigmaticus* from Haweswater (n = 14), Ullswater (n = 4), syntypes of unknown origin (n = 3), and specimens of putative *C. pennantii* from Llyn Tegid (n = 21). Measurements and counts were recorded as described previously.

2.3.5 Data analysis

Individuals of British whitefish from Loch Lomond, Loch Eck, Loch Sloy, Carron Valley Reservoir (putative *C. clupeoides*), Brotherswater, Haweswater, Red Tarn, Ullswater (putative *C. stigmaticus*) and Llyn Tegid (putative *C. pennantii*) were separated using the identification key provided by Kottelat & Freyhof (2007) (Table 2.3). It was found that there was some overlap between separating characters in the key; moreover measurements were sometimes outside the range of the character specified, with a relatively small number of fish being able to be identified without ambiguity (Table 2.4.). Therefore, a set of standard rules to identify the fish was established. If the two outcomes of a dichotomy are A and B, each character within the dichotomy can be categorised as: a, b, x (overlap), a+, b+ (outside range), or missing. Values outside the range of the character are included in the category closest to the value (e.g. a+ = a). In any dichotomy, a missing or overlapping value accompanied by at least one value within a category is classed as that category outcome (e.g. a + x = A). If two values are from opposing categories (e.g. a + b), the fish is classed as unknown and is moved to the next dichotomy. This provides maximum scope for the identification of individual fish. The percentages of no identification possible, correct identification and incorrect identification were examined. The error rate of key and storage method was tested with χ^2. A General Linear Model (GLM) was used to identify what factors from putative species, population nested within putative species and storage method (fresh, frozen, preserved) best explained the variation observed in identification success.

Table 2.4. **The percentages of putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii* that show characters found in dichotomies 3 and 4 which define these species**

		putative *C. stigmaticus* (N = 98)	putative *C. clupeoides* (N = 384)	putative *C. pennantii* (N = 62)
Dichotomy 3 *C. stigmaticus* characters	spots present only	95.9 %	97.9 %	95 %
	IODxHL 3.0 - 3.3 only	22.4 %	40.4 %	65 %
	all characters	20.4 %	39.1 %	61 %
Dichotomy 4 *C. clupeoides* characters	ScLP 7.5-8.5 only	64.3 %	21.9 %	29 %
	ScC 20-21 only	13.3 %	31.8 %	36 %
	AFR 9-11.5 only	39.8 %	39.6 %	21 %
	two characters	38.8 %	28.9 %	18 %
	all characters	1.0 %	3.4 %	0 %

A Kruskal-Wallis test was used to check for differences in the examined characters between putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*. Mann-Whitney U and Fisher's exact tests were used to further examine differences between populations of the same and different putative species. In order to infer how populations were grouped, hierarchical cluster analysis was performed on the mean values of the characters used to separate putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii* for each population.

Principal Components Analysis (PCA) was used to combine all the characters used to separate putative *C. clupeoides*, putative *C. stigmaticus* and putative *C. pennantii* and the differences in PCA scores at a putative species and population level were examined using a GLM. Discriminant Function Analysis (DFA) was used to examine the differences in discriminant function (DF) scores at both putative species and population level when all characters used to separate putative *C. clupeoides*, putative *C. stigmaticus* and putative *C. pennantii* were combined. All statistical analyses were performed using SPSS v. 13.

2.4 RESULTS

Separation 1: *C. pollan* from remaining British whitefish

All of the individuals in the populations examined had sub-terminal mouths (Table. 2.3.) and none were classified as *C. pollan*, therefore there was no error for separation 1, and there was no difference between populations. No individual could be confidently identified as *C. pollan*, thus 544 of 544 individuals were categorised as 'other British whitefish' and were moved to the next level of separation.

Separation 2: *C. oxyrinchus* from remaining British whitefish

C. oxyrinchus are separated from the remaining British whitefish on three criteria: lacustrine/anadromous, eye diameter times in snout length (EDxSL), gill raker number (GR) (Table. 2.3.). In this study 100 % the individuals examined were lacustrine, and 99.8 % (1 missing data) of the individuals had a EDxSL less than 2, therefore of 544 fish, 543 could not be assigned to *C. oxyrinchus* based on these two characters. However, for GR there was allocation overlap, individuals could be potentially assigned to three categories: 1) in or about *C. oxyrinchus* or 2) in or about remaining British whitefish, or 3) into the overlap which could fall into either category. From a total of 396 fish for which GR counts were available, 0.5 % were assigned to *C. oxyrinchus*, 39.4 % were assigned to the overlapping GR count, and 60.1 % were assigned to remaining British whitefish. No individual could be confidently identified as *C. oxyrinchus*, thus 544 of 544 individuals

were categorised as ‘other British whitefish’ and were moved to the next level of separation.

Separation 3: *C stigmaticus* from remaining British whitefish

*C stigmaticu*s are separated from the remaining British whitefish on two criteria: presence of spots and interorbital distance times in head length (IODxHL) (Table. 2.3.). In this study most of the individuals examined had small blackish spots on the flank; using this character, individuals could be potentially assigned to two categories: 1) *C. stigmaticu*s or 2) remaining British whitefish. From a total of 544 fish, 97.2 % were assigned to *C. stigmaticu*s and 2.8 % were assigned to remaining British whitefish. There was not a significant difference in the presence of spots between putative *C. stigmaticus* and the remaining British whitefish (Fisher’s exact test, d.f. = 1, p = 0.33). For IODxHL there was a small allocation overlap, where individuals could be potentially assigned to one of three categories: 1) in or about *C. stigmaticu*s or 2) in or about remaining British whitefish, or 3) into the overlap which could fall into either category. From a total of 544 fish, 46.5 % were assigned to *C. stigmaticu*s, 12.7 % were assigned to the overlapping IODxHL measurement and 40.8 % were assigned to remaining British whitefish. There was a significant difference in IODxHL between putative *C. stigmaticus* and the remaining British whitefish (Mann-Whitney U test, d.f. = 1, p < 0.0001). Of 544 individuals, 311 could be confidently identified as *C. stigmaticu*s, thus 233 individuals were categorised as ‘other British whitefish’ and were moved to the next level of separation.

Separation 4: *C. clupeoides* from *C. pennantii*

C. clupeoides are separated from *C. pennantii* by three criteria: scale rows between the lateral line and pelvic fin base (ScLP), circumpeduncular scale rows (ScC) and number of branched rays on the anal fin (AFR) (Table. 2.3). For ScLP there was a small allocation overlap, individuals could be potentially assigned to three categories: 1) in or about *C. clupeoides* or 2) in or about *C. pennantii*, or 3) into the overlap which could fall into either category. From a total of 219 fish for which this scale count was available, none were assigned to *C. clupeoides*, 25.1 % were assigned to the overlapping ScLP count, and 74.9 % were assigned to *C. pennantii*. There was no significant difference in the number of ScLP between putative *C. clupeoides* and putative *C. pennantii* (Mann Whitney-U, d.f. = 1, p = 0.06). Using ScC individuals could be potentially assigned to two categories: 1) in or about *C. clupeoides* or 2) in or about *C. pennantii*. From a total of 228 fish for which this scale count were available, 32.5 % were assigned to *C. clupeoides* and 67.5 % were assigned to *C. pennantii*. There was a significant difference in ScC between putative *C.*

clupeoides and putative *C. pennantii*. (Mann Whitney-U, d.f. = 1, $p < 0.05$). Using AFR there was a small allocation overlap, individuals could be potentially assigned to three categories: 1) *C. clupeoides* or 2) *C. pennantii* with certainty, or 3) into the overlap which could fall into either category. From a total of 230 fish for which AFR counts were available, 6.1 % were assigned to *C. clupeoides*, 35.2 % were assigned to the overlapping AFR, and 58.7 % were assigned to *C. pennantii*. There was a significant difference in AFR between putative *C. clupeoides* and putative *C. pennantii* (Mann Whitney-U, d.f. = 1, $p < 0.001$). Of 233 individuals, two could be confidently identified as *C. clupeoides*, and 138 could be confidently identified as *C. pennantii*, thus 93 individuals were categorised as 'unidentified British whitefish'.

Table 2.5 The percentages of putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii* from nine UK populations assigned to categories: unidentified, powan, schelly and gwyniad. Shaded areas show correct allocations. Bold values indicate which species fish are most commonly identified as

Key assignment	putative *C. clupeoides*				putative *C. stigmaticus*				putative *C. pennantii*
	Lomond	Eck	Sloy	Carron Valley	Brotherswater	Haweswater	Red Tarn	Ullswater	Llyn Tegid
C. clupeoides	0 %	0.7 %	1.4 %	0 %	0 %	0 %	0 %	0 %	0 %
C. stigmaticus	**55.0 %**	25.7 %	**42.0 %**	**66.1 %**	**100 %**	**92.0 %**	**100 %**	**93.9 %**	**77.4 %**
C. pennantii	30.0 %	**41.9 %**	27.5 %	23.7 %	0 %	8.0 %	0 %	3.0 %	14.5 %
Unidentified	15.0 %	31.6 %	29.0 %	10.2 %	0 %	0 %	0 %	3.0 %	8.1 %

Of 544 individual fish analysed using the classification criteria of Kottelat & Freyhof (2007), only 105 were identified correctly, while 346 were identified incorrectly and 93 were classified as unidentified British whitefish. While putative *C. clupeoides* (76.8 %) and putative *C. pennantii* (77.4 %) were most often identified incorrectly, putative *C. stigmaticus* (95.9 %) were most often identified correctly. There is a significant difference between the accuracy of identification between putative *C. clupeoides*, putative *C. stigmaticus* and putative *C. pennantii* (χ^2, d.f. = 4, $p<0.0001$). Fish from all populations were most often identified as *C. stigmaticus*, apart from putative *C. clupeoides* from Loch Eck which were most often identified as *C. pennantii* (Table 2.5). The key was the least successful for the examined individuals of putative *C. clupeoides* (Table 2.5). The effect of storage method on the result (unidentified, correct, incorrect) was tested within each putative species. There was no significant difference in number allocated to each result depending on the storage method (fresh, frozen, alcohol) for putative *C. clupeoides* (χ^2, d.f. = 4, $p = 0.55$), putative *C. stigmaticus* (χ^2, d.f. = 4, $p = 0.25$), and putative *C. pennantii* (χ^2, d.f. = 2, $p = 0.86$). A GLM was used to identify what factors best explained the variation observed in identification success, population nested within putative species ($F_{6,533} = 3.3$, p

< 0.005) and putative species ($F_{2,533}$ = 293.3, p < 0.0001) could significantly explain the variation, however, storage method could not ($F_{2,533}$ = 1.6, p = 0.21).

Significant differences were found between putative *C. clupeoides*, putative *C. stigmaticus* and putative *C. pennantii* for most of the characters examined: eye diameter times in snout length (Kruskal-Wallis, d.f. = 2, p < 0.0001) (Fig. 2.1.), gill raker number (Kruskal-Wallis, d.f. = 2, p < 0.0001) (Fig. 2.2.), interorbital distance times in head length (Kruskal-Wallis, d.f. = 2, p < 0.0001) (Fig. 2.4.), scale rows from the lateral line to the pelvic fin base (Kruskal-Wallis, d.f. = 2, p < 0.0001) (Fig. 2.5.), circumpeduncular scale rows (Kruskal-Wallis, d.f. = 2, p < 0.0001) (Fig. 2.6.), and anal fin rays (Kruskal-Wallis, d.f. = 2, p < 0.01) (Fig. 2.7.), but not for spots (χ^2, d.f. = 2 p = 0.32) (Fig. 2.3.).

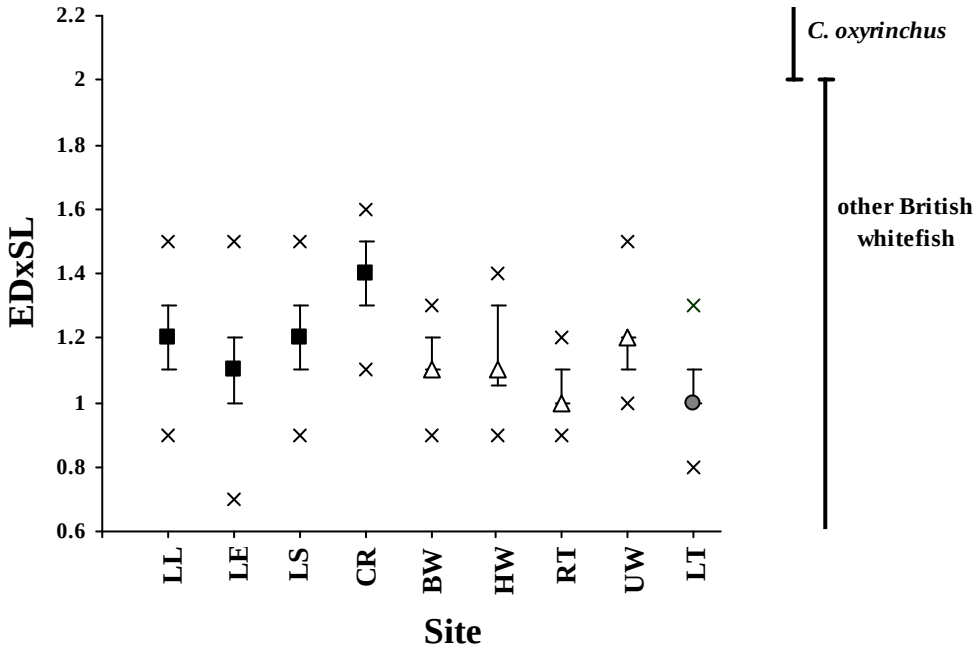


Figure 2.1 Median eye diameter times in snout in populations if British whitefish. LL = Loch Lomond, LE = Loch Eck, LS = Loch Sloy, CR = Carron Valley Reservoir, BW = Brotherswater, HW = Haweswater, RT = Red Tarn, UW = Ullswater, LT = Llyn Tegid. Black markers: putative *C. clupeoides*, white markers: putative *C. stigmaticus*, grey marker: putative *C. pennantii*; lower bars indicate 25 % percentile, upper bars indicate 75 % percentile; X indicates range. Range of value for species in key indicated.

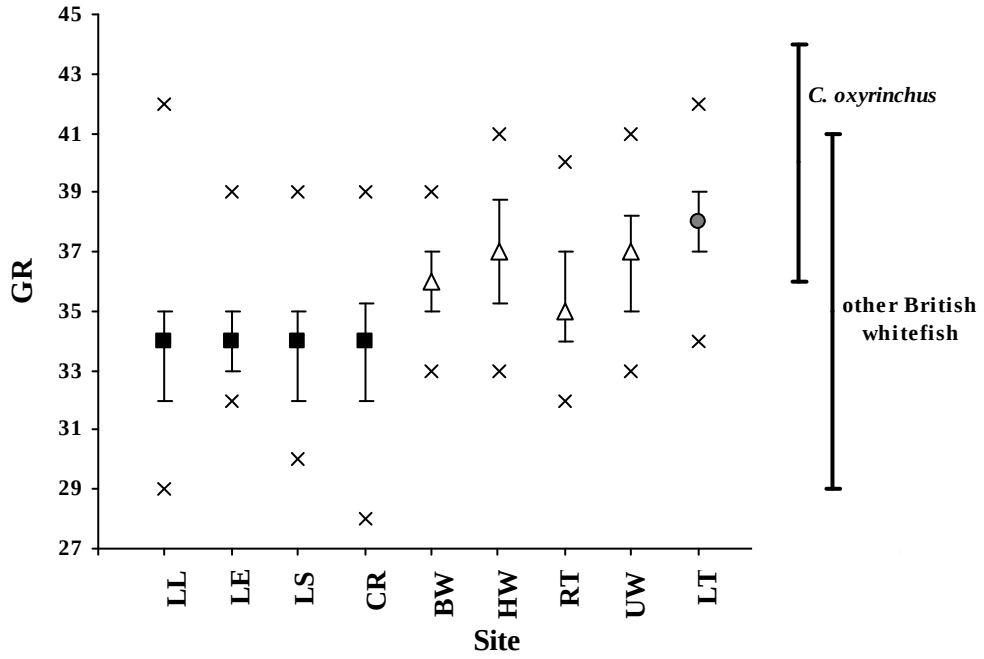


Figure 2.2 Median gill raker number in populations of British whitefish

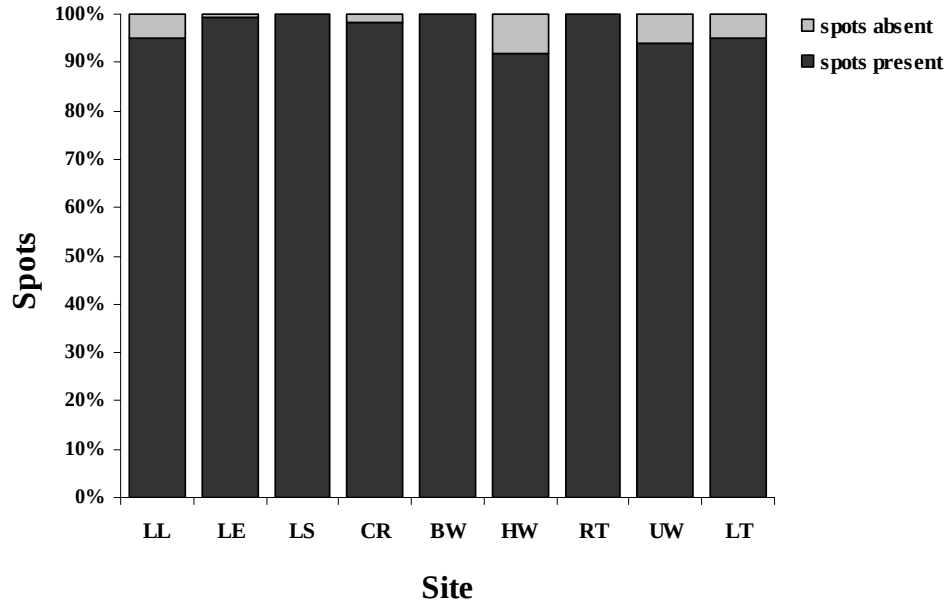


Figure 2.3 Proportionate frequency of individuals with presence of spots in populations of putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*.

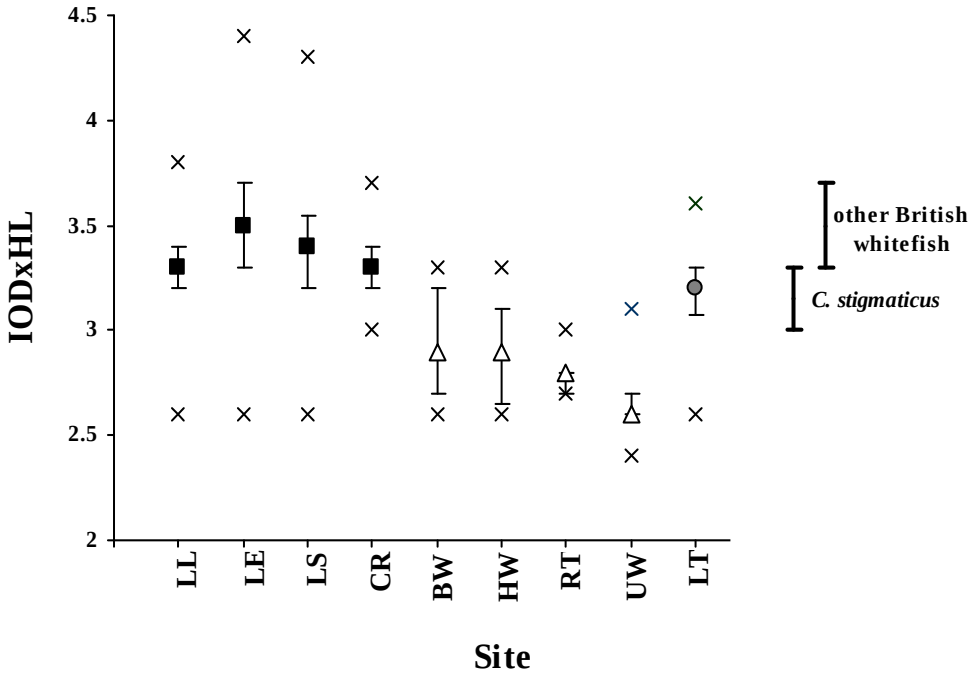


Figure 2.4 Median interorbital distance x in head length in populations of British whitefish

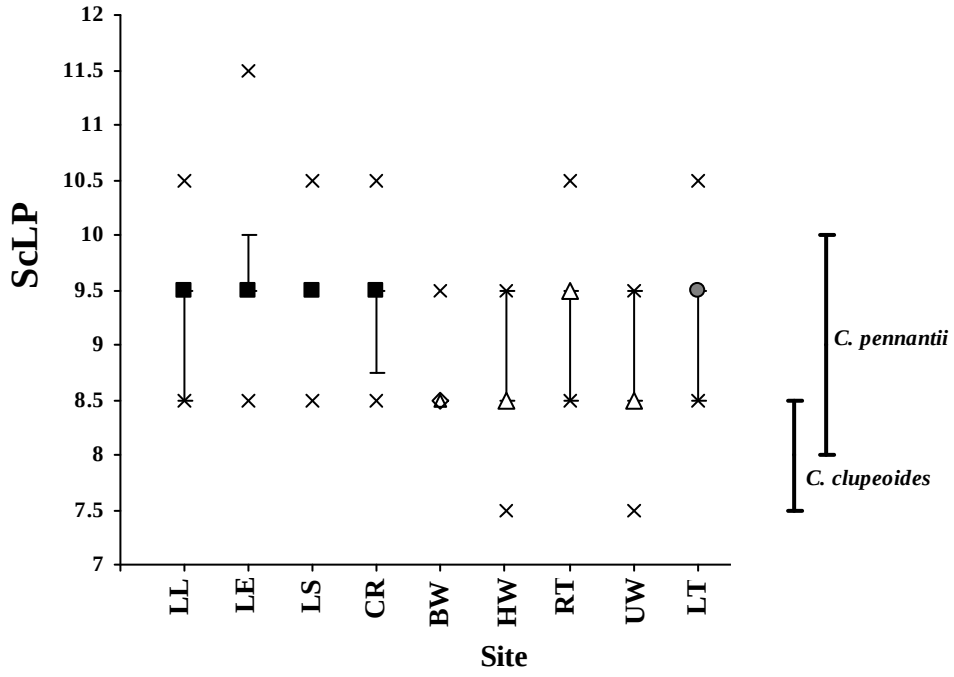


Figure 2.5 Median scale rows from the lateral line to the pelvic fin base in populations of British whitefish

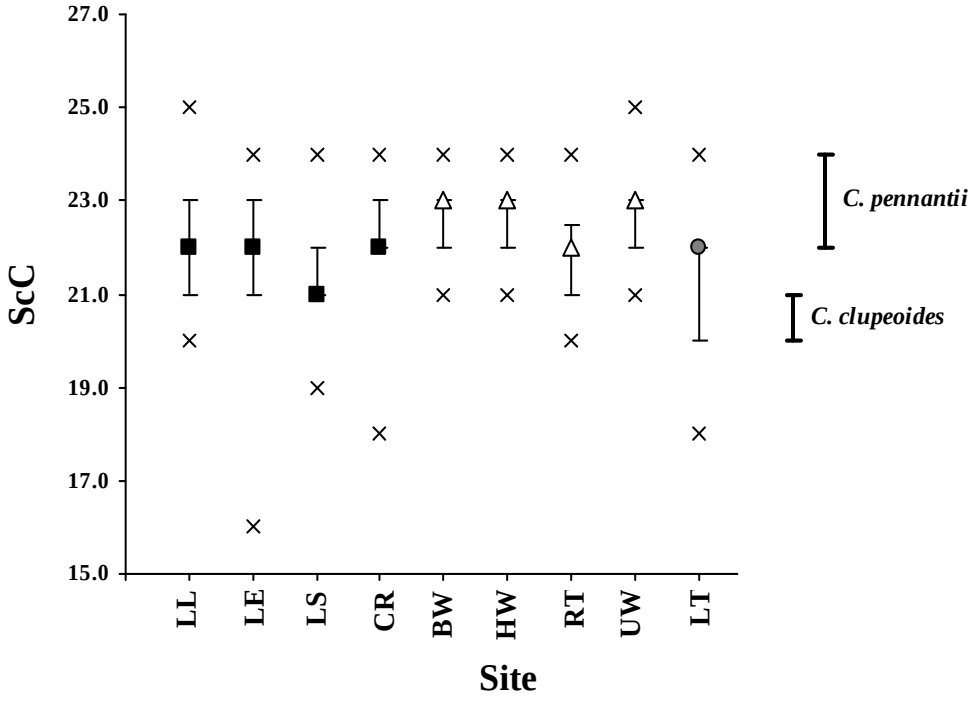


Figure 2.6 Median circumpeduncular scale rows in populations of British whitefish

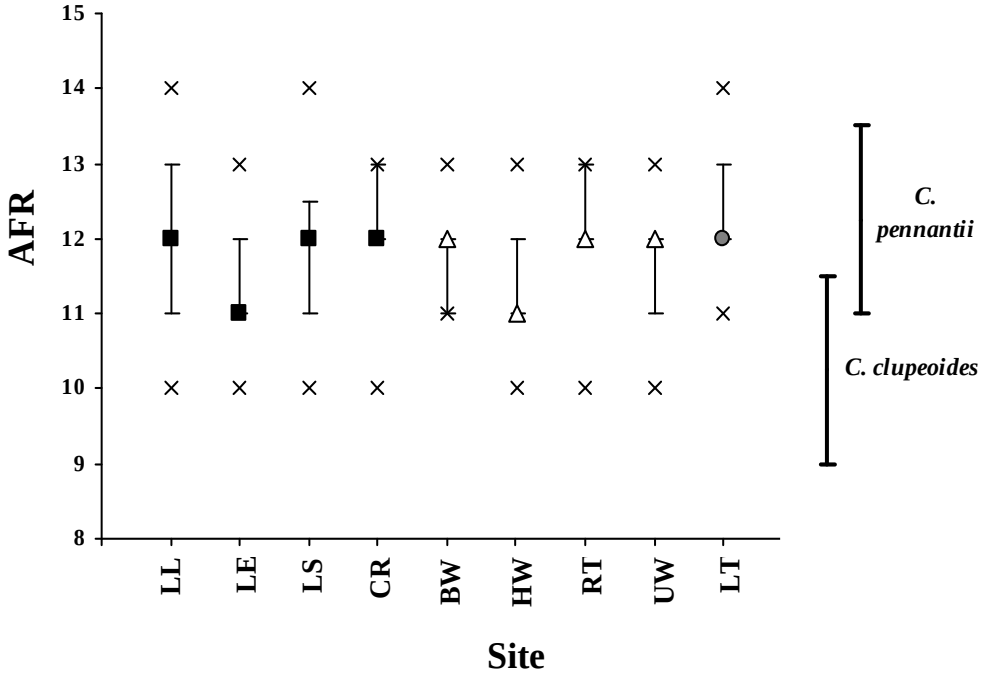


Figure 2.7 Median anal fin rays in populations of British whitefish.

In comparisons of characters examined between populations of different putative species in the relevant separations only 40.4 % were found to be significantly different (Bonferroni corrected, $p < 0.001$) (shaded areas, Table 2.6.). Of comparisons between populations of the same putative species in the relevant separations 23.3 % were also significantly different (Bonferroni corrected, $p < 0.002$) (shaded areas, Table 2.7.). In the examined characters, the two most similar populations of the same putative species were Loch Lomond and Carron Valley Reservoir (the most non-significant in Table 2.7, and most similar in Fig. 2.8), which are source and refuge population respectively, while the most different were Loch Lomond and Loch Eck.

Table 2.6 p values calculated in a comparison of median values between populations of different putative species (Mann-Whitney U, d.f. = 1). Bonferroni corrected, significance indicated by p < 0.001. LL = Loch Lomond, LE = Loch Eck, LS = Loch Sloy, CR = Carron Valley Reservoir, BW = Brotherswater, HW = Haweswater, RT = Red Tarn, UW = Ullswater, LT = Llyn Tegid. A = putative *C. clupeoides* and *C. stigmaticus*, B = putative C. *clupeoides* and *C. pennantii*, C = putative *C. stigmaticus* and *C. pennantii*; bold type indicates no significant difference; shaded regions indicate comparisons between these putative species in the key (dichotomy 3 and 4).

	Population comparison		Spots	IODxHL	ScLP	ScC	AFR
A	LL	BW	**NS**	< 0.0001	< 0.0002	**NS**	**NS**
	LL	HW	**NS**	< 0.0001	< 0.001	**NS**	**NS**
	LL	RT	**NS**	< 0.0001	**NS**	**NS**	**NS**
	LL	UW	**NS**	< 0.0001	< 0.001	< 0.0002	**NS**
	LE	BW	**NS**	< 0.0001	< 0.0001	< 0.001	**NS**
	LE	HW	**NS**	< 0.0001	< 0.0001	< 0.001	**NS**
	LE	RT	**NS**	< 0.0001	< 0.001	**NS**	< 0.0001
	LE	UW	**NS**	< 0.0001	< 0.0001	< 0.0001	**NS**
	LS	BW	**NS**	< 0.0001	< 0.0001	< 0.0001	**NS**
	LS	HW	**NS**	< 0.0001	< 0.0001	< 0.0002	**NS**
	LS	RT	**NS**	< 0.0001	**NS**	**NS**	**NS**
	LS	UW	**NS**	< 0.0001	< 0.0001	< 0.0001	**NS**
	CR	BW	**NS**	< 0.0001	< 0.0001	**NS**	**NS**
	CR	HW	**NS**	< 0.0001	< 0.0002	**NS**	< 0.001
	CR	RT	**NS**	< 0.0001	**NS**	**NS**	**NS**
	CR	UW	**NS**	< 0.0001	< 0.0002	< 0.0001	**NS**
B	LL	LT	**NS**	**NS**	**NS**	**NS**	**NS**
	LE	LT	**NS**	< 0.0001	< 0.001	**NS**	< 0.0001
	LS	LT	**NS**	< 0.0001	**NS**	**NS**	**NS**
	CR	LT	**NS**	**NS**	**NS**	**NS**	**NS**
C	BW	LT	**NS**	**NS**	< 0.0002	< 0.0001	**NS**
	HW	LT	**NS**	< 0.0001	< 0.001	< 0.0001	< 0.0004
	RT	LT	**NS**	< 0.0001	**NS**	**NS**	**NS**
	UW	LT	**NS**	< 0.0001	< 0.001	< 0.0001	**NS**

Table 2.7 p values calculated in a comparison of median values between populations of the same putative species (Mann-Whitney U, d.f. = 1). Bonferroni corrected, significance indicated by p < 0.002. Bold type indicates a significant difference; shaded regions indicate comparisons between populations between source and founded, or between founded populations from same source population.

	Population comparison		Spots	IODxHL	ScLP	ScC	AFR
A	LL	LE	NS	**< 0.0001**	**< 0.0001**	NS	**< 0.0001**
	LL	LS	NS	NS	NS	NS	NS
	LL	CR	NS	NS	NS	NS	NS
	LE	LS	NS	NS	NS	NS	**< 0.0001**
	LE	CR	NS	**< 0.0001**	NS	NS	**< 0.0001**
	LS	CR	NS	NS	NS	NS	NS
B	BW	HW	NS	NS	NS	NS	NS
	BW	RT	NS	NS	NS	NS	NS
	BW	UW	NS	**< 0.0001**	NS	NS	NS
	HW	RT	NS	NS	NS	NS	**< 0.002**
	HW	UW	NS	**< 0.0001**	NS	NS	NS
	RT	UW	NS	**< 0.0001**	NS	**< 0.0003**	NS

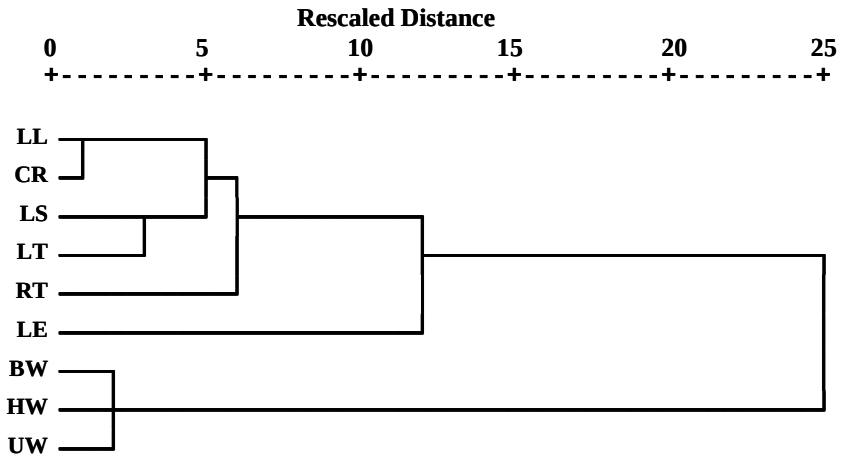


Figure 2.8 Hierarchical cluster analysis dendrogram using Average Linkage (Between Groups), derived from population means of characters in dichotomy 3 and 4 of the key that separate putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*

Using hierarchical cluster analysis on the mean character values for each population it was found that populations did not cluster according to Kottelat & Freyhof's putative species. When two clusters were formed these were divided into 1) Loch Lomond, Loch Eck, Loch Sloy, Carron Valley Reservoir (putative *C. clupeoides*), Red Tarn (putative *C. stigmaticus*) and Llyn Tegid (*putative C. pennantii*), and 2) Brotherswater, Haweswater and Ullswater (putative *C. stigmaticus*). When three clusters were formed these were divided into 1) Loch Lomond, Loch Sloy, Carron Valley Reservoir (putative *C. clupeoides*), Red Tarn (putative

C. stigmaticus) and Llyn Tegid (*putative C. pennantii*), 2) Loch Eck (putative *C. clupeoides*), and 3) Brotherswater, Haweswater and Ullswater (putative *C. stigmaticus*) (Fig. 2.8.). The source population Loch Lomond and the refuge populations of Carron Valley Reservoir clustered together.

Principal component scores were extracted from the all the characters used in separations 3 and 4 in the key. PC1 explained 25.1 % of the total variation in the examined characters. Individuals with an extreme positive PC1 score were typified by a high IODxHL, a high ScLP and a low presence of spots. PC2 explained 21.9 % of the total variation in the examined characters. Individuals with an extreme positive PC2 score were typified by a high ScC, a high AFR, and a low IODxHL (Fig. 2.9. & 2.10.).

GLM analysis of principal component scores of characters that separate *C. clupeoides*, *C. stigmaticus* and *C. pennantii* using the factors putative species and putative species(population), indicated that while most of the variation observed (Partial Eta squared = 0.24) was explained by putative species ($F_{2,365} = 79.6$, $p < 0.0001$), a significant though lesser amount (Partial Eta squared = 0.13) was explained by population nested within species ($F_{6,365} = 11.9$, $p < 0.0001$).

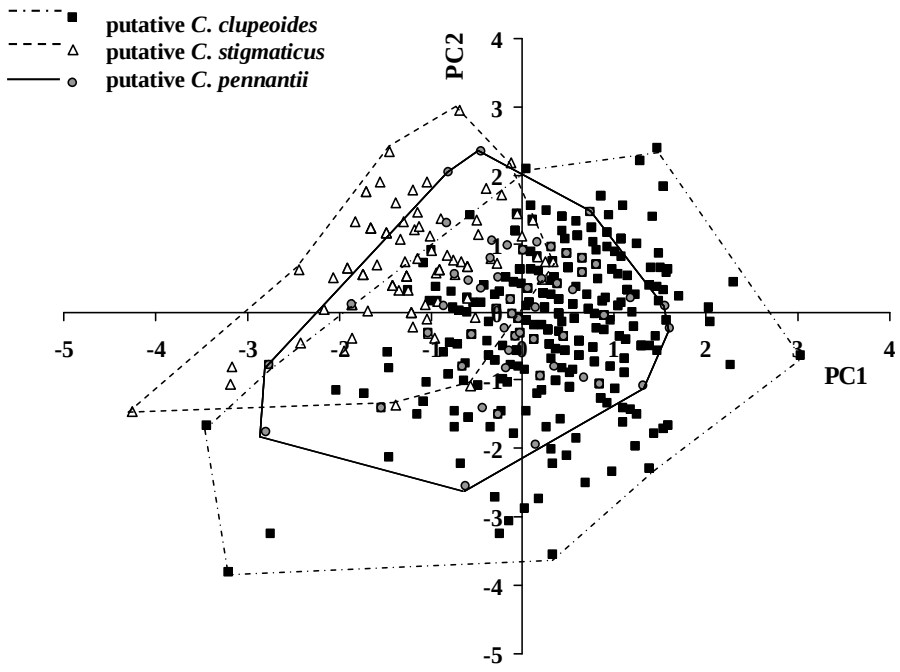


Figure 2.9 PCA scores derived from characters in dichotomy 3 and 4 of the key that separate putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*. Limit of each putative species indicated by an unbroken or dotted line

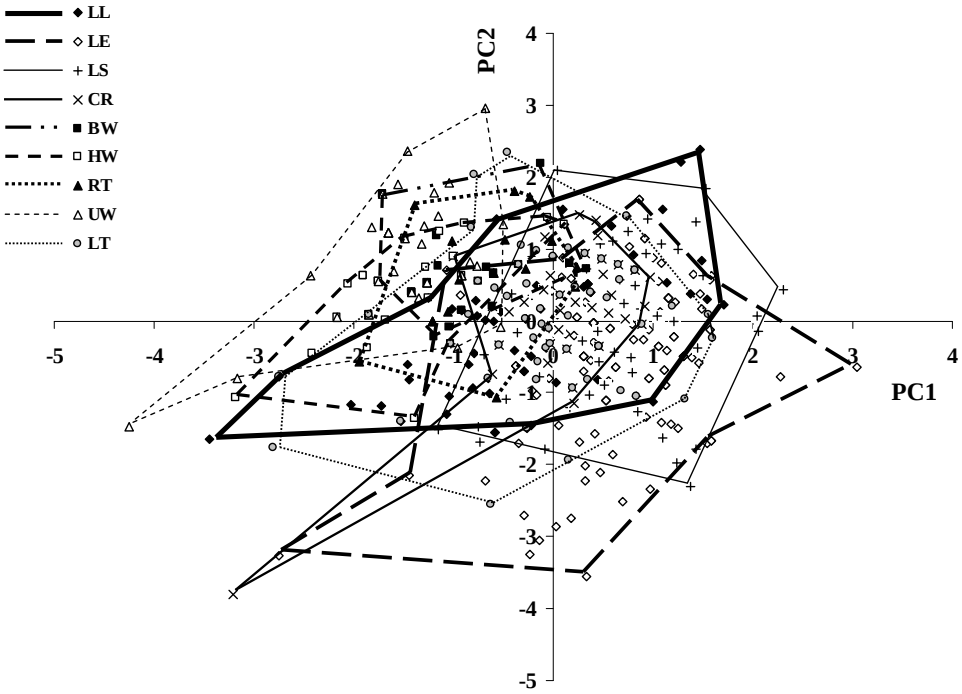


Figure 2.10 Population PCA scores derived from characters in dichotomy 3 and 4 of the key that separate putative *C. clupeoides, C. stigmaticus* and *C. pennantii*. Different markers indicate different UK populations, limit of each population indicated by an unbroken or dotted line

Discriminant analysis of putative species revealed two discriminant functions (DFs). The first explained 94.3 % of the variance (canonical $R^2 = 0.52$), the second explained 5.7 % of the variance (canonical $R^2 = 0.06$). Together these two DFs significantly differentiated the populations ($\chi^2 = 390.5$, d.f. = 10, $p < 0.0001$). The correlations between characters and DFs indicated that IODxHL (r = 0.83) had the most important contribution to DF1 which separated putative *C. clupeoides* and putative *C. pennantii* from putative *C. stigmaticus*. AFR (r = 0.65) had the most important contribution to DF2 which separated putative *C. pennantii* from putative *C. stigmaticus* and putative *C. clupeoides*. Classification results were good for putative *C. clupeoides* and putative *C. stigmaticus* with 94.2 % and 81.3 % allocated to the correct group respectively, however only 3.4 % of putative *C. pennantii* were allocated to the correct group, with most (83.1 %) allocated to the *C. clupeoides* group (Fig. 2.11.).

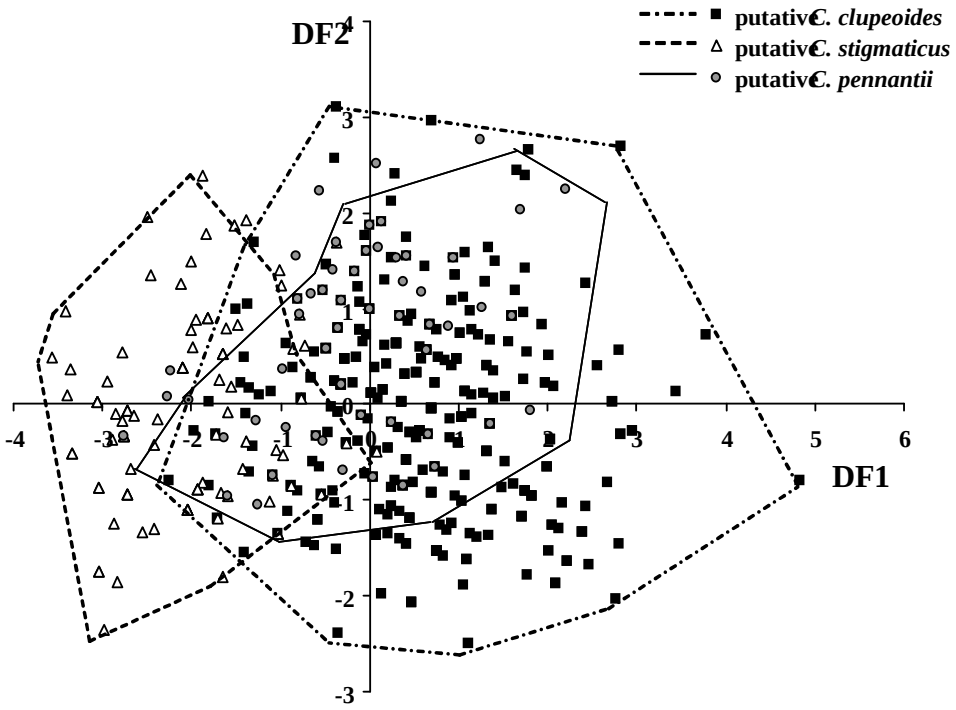


Figure 2.11 DF scores derived from characters in dichotomy 3 and 4 of the key that separate putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*. Limit of each putative species indicated by a line

A discriminant analysis of different UK whitefish populations revealed five discriminant functions (DFs). The first explained 86.9 % of the variance (canonical $R^2 = 0.63$), the second explained 8.8 % of the variance (canonical $R^2 = 0.15$), the third explained 3.0 % of the variance (canonical $R^2 = 0.06$). Together these DFs significantly differentiated the populations ($\chi^2 = 603.3$, d.f. = 40, $p < 0.0001$). The correlations between characters and DFs indicated that IODxHL (r = 0.75) had the most important contribution to DF1 which separated Loch Eck, Loch Sloy, Loch Lomond, Carron Valley Reservoir and Llyn Tegid fish from Ullswater, Haweswater, Red Tarn and Brotherswater fish. AFR (r = 0.89) had the most important contribution to DF2 which separated Llyn Tegid, Red Tarn, Carron Valley Reservoir, Loch Sloy and Loch Lomond fish from Haweswater, Loch Eck, Brotherswater, and Ullswater, fish. IODxHL (r = 0.66) had the most important contribution to DF3 which separated Brotherswater Carron Valley Reservoir, Loch Lomond Haweswater Loch Sloy fish from Red Tarn, Ullswater, Llyn Tegid and Loch Eck fish. Classification results were best for Loch Lomond (63.6 %), Loch Eck (76.0 %), Red Tarn (52.4 %) and Ullswater (72.7 %) in which most individuals were allocated to the correct group. However for Loch Sloy only 4.5 % were allocated correctly and 51.5 % were allocated to Loch Lomond; for Carron Valley Reservoir none were allocated correctly and 73.1 % were allocated to Loch

Lomond; for Brotherswater none were allocated correctly and 36.8 % were allocated to Loch Lomond; for Haweswater only 17.4 % were allocated correctly and 39.1 % were allocated to Ullswater, and for Llyn Tegid only 27.1 % were allocated correctly and 42.4 % were allocated to Loch Lomond (Fig. 2.12.).

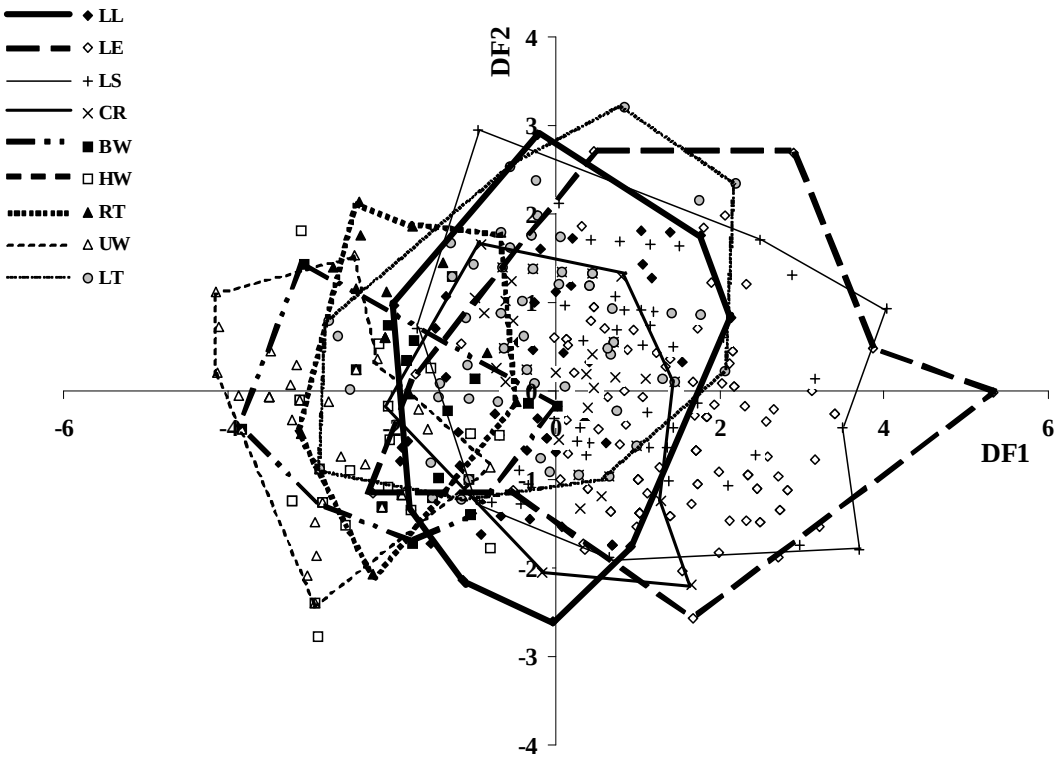


Figure 2.12 DF scores derived from characters in dichotomy 3 and 4 of the key that separate putative *C. clupeoides, C. stigmaticus* and *C. pennantii*. Different markers indicate different UK populations, limit of each population indicated by a line

2.5 DISCUSSION

The dichotomous key defining putative whitefish species (Kottelat & Freyhof, 2007) performed poorly. This indicated that the key had little power to discriminate individual British whitefish to putative species defined by Kottelat & Freyhof (2007), with an over all success rate of only 19 %. While dichotomies 1 and 2 resulted in no incorrect identification, these dichotomies separated fish that are currently recognised as different species in Britain from others in the genus. Dichotomy 3, theoretically separating *C. stigmaticus* from other British whitefish, was unsuccessful. There were 98 putative *C. stigmaticus*; 311 *C. stigmaticus* were identified, of these 94 were correct. Dichotomy 4 was also unsuccessful. There were 384 putative *C. clupeoides*; 2 *C. clupeoides* were identified, of these 2 were correct, and there were 62 putative *C. pennantii*; 138 *C. pennantii* were

identified, of these 9 were correct. Putative species allocation error was not due to storage method.

Regardless of the statistical analysis employed here, the whitefish populations could not be separated into the three putative species as designated by Kottelat & Freyhof (2007). Cluster analysis of populations into three groups did not result in a split into the three putative species. Conversely one cluster was composed of a mixture of putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii* populations, a second was composed solely of Loch Eck putative *C. clupeoides*, whilst a third was composed of three putative *C. stigmaticus* populations. This latter result suggests that Kottelat & Freyhof's key had some discriminatory capacity to separate putative *C. stigmaticus* from the other putative species. The GLM analysis suggests the populations cannot be separated with great confidence into these putative species: this was supported by the large overlap between putative species and populations in these characters. While a greater amount of variation was explained by putative species (24 %), the variation between populations excluding that explained by putative species was also highly significant (13 %). Using DFA, the most reliable separation was for putative *C. clupeoides*; conversely this putative species was the least well differentiated by the key. However, the separation was not complete and there was very significant overlap between individuals of all putative species. The population level DFA also showed overlap between all populations. In an analysis that maximises the differentiation between groups this was not indicative of well-differentiated species.

In addition, it was found that putative *C. clupeoides* originating from Loch Lomond, but transferred to refuge sites were very similar to each other and Loch Lomond fish, but were not identical. Carron Valley Reservoir fish clustered with Loch Lomond in a cluster analysis, and in the population level DFA were always allocated as Loch Lomond fish. However, in the cluster analysis Loch Sloy putative *C. clupeoides* tended to cluster with Llyn Tegid putative *C. pennantii*. There were also significant differences between Loch Lomond and Loch Sloy fish in PC1 of the PCA, and between Loch Sloy and Loch Lomond and between Loch Sloy and Carron Valley Reservoir fish in the DFA. Putative *C. clupeoides* from Loch Lomond and Loch Eck, which are believed to have been separated for thousands of years, were the most different native populations of the same putative species.

When characters between populations of different putative species were compared, unexpectedly no significant differences were found in some characters. IODxHL

performed well in separating putative *C. stigmaticus* populations from putative *C. clupeoides* and *C. pennantii* populations, however, the presence or absence of spots performed badly in the same comparison. The characters used to separate putative *C. clupeoides* and putative *C. pennantii* populations typically displayed no significant differences between populations e.g. ScC did not differ significantly between these populations. When characters between populations of the same putative species were compared, unexpectedly significant differences were found between some characters. In this analysis, the character spots performed well, with no significant differences between putative *C. stigmaticus* populations, however, IODxHL was significant in half of all comparisons. ScC performed best in terms of comparison between putative *C. clupeoides* populations with no evidence for significant differences, but AFR differed in half the comparisons. This reveals an overall poor performance by those characters selected by Kottelat & Freyhof (2007) since a reliable defining character should discriminate between populations of different species but not between populations of the same species.

There are three possible reasons for the inability of the key published by Kottelat & Freyhof (2007) to discriminate putative Coregonid species: 1) incorrect use of the key (human error), 2) poor characters used to separate these putative species (possibly due to plasticity, homology or few descriptive specimens), 3) Kottelat & Freyhof's three putative species are not robust species as suggested. Error in the use of the key is possible; however it is thought to be unlikely. Firstly counts and measurements were taken as instructed by Kottelat & Freyhof (2007) and secondly, as much leeway as possible was given to allow identification of specimens. Human error on its own cannot therefore justify such a low success rate, a species key should be usable by fish biologists, not just by taxonomic specialists and as such the other two options are discussed.

Many postglacial fishes are phenotypically plastic (Skulason & Smith, 1995) and whitefish species are known to exhibit homoplasy for morphological traits (Bernatchez *et al.*, 1996; Douglas *et al.*, 2005; Ostbye *et al.*, 2005a, 2006; Hansen *et al.*, 2008). Thus some characters may either reflect differences between species, or a response to abiotic and biotic pressures spatially and temporally (Svärdson, 1951; Lindsey, 1981; Swain & Lindsey, 1986). Characters in postglacial fishes known to respond plastically to environmental pressures include those with high functional significance such as head features that involve the capture and handling of food (Garduño-Paz, 2009). This ability for plastic change in British whitefish has been demonstrated in a study comparing source and refuge populations of putative *C. clupeoides* (Chapter 4). Certainly the differences

found between Loch Lomond putative *C. clupeoides* and Loch Sloy putative *C. clupeoides* (originating from Loch Lomond) suggest that at least some of the characters used in the key are plastic.

In addition, the choice of taxonomic characters was based on few individuals in historical species descriptions. For these populations Kottelat & Freyhof (2007) have apparently followed Tate Regan (1911) though not for putative *C. vandesius* or putative *C. pollan* which were each divided into two species by Tate Regan. Many historical species descriptions rely on only a few specimens e.g. in LaCepède (1803) *C. clupeoides* was described from a second-hand account of "several" Loch Lomond whitefish; Tate Regan (1908, 1911) described *C. stigmaticus* from 13 specimens from unknown location(s). It has been suggested that many more specimens (50 +) are required in order be confident that a few individuals that share one character state are not drawn from populations that actually consist of more than one morphotype (Walsh, 2000).

The other possible reason for the poor performance of Kottelat & Freyhof's key was that the three putative species recognised by Kottelat & Freyhof (2007) are not 'good' species: groups that show substantial reproductive isolation from other such groups (Coyne & Orr, 2004). There is clearly variation amongst and within populations. This could be explained, as Kottelat & Freyhof (2007) suggest, by multiple species that show little variation, however, if the key was considered to be correct most populations contain more than one putative species. Though this does not follow the Kottelat & Freyhof (2007) putative species classification, this could be due to introgression after secondary contact. In Loch Lomond however, no genetic sub-structuring has been found and this appears to be a monomorphic population of whitefish (Thompson *et al.*, 2008; Chapter 5). It appears more likely that these differences reflect natural variation in populations and the variation seen is due to one highly variable species.

There have been some taxonomic and genetic examinations of the systematics of UK whitefish populations. Biochemical studies have resulted in a range of conflicting results from no differentiation between populations (Ferguson, 1974), to differentiation between putative *C. stigmaticus* populations and other populations (Hartley, 1995), and differentiation between putative *C. pennantii* from other populations (Bridges & Yoshikami, 1970; Beaumont *et al.*, 1995). Reciprocal crossing experiments between Loch Lomond putative *C. clupeoides* and putative *C. pennantii* point to some incompatibility (Haram, 1968). Recent preliminary genetic work indicates the evolutionary relationship of

Loch Lomond putative *C. clupeoides* to putative *C. pennantii* is closer than either is to putative C. *vandesius* (Thompson *et al.,* 2008). It is hoped that in the future additional more detailed taxonomic and genetic work will be completed on all of the UK populations. However, from the results presented here, it is apparent that there is currently little support for the definition of three putative species amongst UK populations of *C. lavaretus* suggested by Kottelat & Freyhof (2007). The high degree of variation between and within populations is not clearly apportioned into three clear species, but is more likely the result of a single highly variable species where variability is the result of plasticity, founder effects and adaptation.

Conservation biologists are some of the main users of species level taxa, but participate relatively little in the debate over conservation implications of taxonomic changes (Rojas, 1992; but see Agapow *et al.,* 2004; Mina *et al.,* 2006). It should be noted that the IUCN website (IUCN, 2009) already recognises Kottelat & Freyhof's putative species and has done so without any discussion or agreement amongst UK fish biologists or conservation agencies. This unilateral acceptance of a new taxonomy is likely to result in significant shifts in how fishes are recognised, especially by non-experts (e.g. funding bodies, conservation pressure groups). It is unlikely the repeated changing of names without verification is useful and may actually impinge on conservation actions. At present *C. lavaretus* is protected under Schedule 5 of the Wildlife and Countryside Act (1981) and features as a priority species in the UK Biodiversity Action Plan (UKBAP); *C. clupeoides*, *C. stigmaticus* and *C. pennantii* do not. With ambiguity in their species designation, in theory, these populations currently have no legal protection. While an increase in the conservation status of UK whitefish (i.e. to endemic species, c.f. rare British populations of a pan-European species) is to be welcomed, whether this would translate into more resources is arguable with the addition of many more endemic British fish species according to Kottelat & Freyhof (2007), e.g. Arctic charr *Salvelinus alpinus* which shifts from one species to 11 putative species. It is agreed with Kottelat & Freyhof that conservation should be focussed on the populations (as they are clearly different) but there is little statistical support for these 'species'. Current management in the UK effectively treats each whitefish population as a separate evolutionary significant unit (ESU). As such this allows the populations to be conserved and resources allocated depending on phenotypic, ecological and genetic distinctiveness.

Kottelat & Freyhof (2007) made several wide-ranging changes to the systematics of the UK and European ichthyofauna. They recognised that in some cases, the keys presented in

their *Handbook of European Freshwater Fishes* may be incorrect; it is clear that in this case of the UK European whitefish, I must agree. The allocation of British whitefish populations into three species as proposed by Kottelat & Freyhof (2007) is not supported by evidence available to date. The view that these populations remain as the single but variable species *C. lavaretus* (which importantly retains existing legal protection) is strongly supported, until and unless more detailed taxonomic evidence supports a different view. It is also recommended that other 'species' should receive similar levels of scrutiny before any changes are made to the currently accepted UK species list.

Chapter 3 DISCONTINUOUS INFRA-SPECIFIC VARIATION IN ECOLOGICAL AND MORPHOLOGICAL TRAITS HAVE CONSEQUENCES FOR CONSERVATION OF POWAN (*COREGONUS LAVARETUS*) IN SCOTLAND[1]

3.1 ABSTRACT

In the UK, legislation concerning wildlife mainly affords protection at species level excluding a few sub-species of birds and hybrids of plants. However, this does not take into account conservation of biodiversity at an infra-species level. Different populations of the same species in some cases are ecologically or genetically distinct and thus have different conservation needs. These may need to be managed separately as opposed to different populations of a species being managed identically. This is illustrated by examining two populations of powan (*Coregonus lavaretus*) found in Scotland. Significant morphological and ecological differences were found between these two populations, suggesting that they are not ecologically exchangeable and form evolutionary significant units (ESUs). In order for this to be accepted, guidelines that recognise infra-specific level protection and management need to be implemented.

3.2 INTRODUCTION

The conservation and management of organisms in the wild necessitates a concept of identifiable units which require monitoring, conservation, management or protection. Without this, it is impossible to define conservation need, develop management strategies, legislate, determine management outcomes, develop policy or demonstrate change. By far the most important and widely used biological unit in conservation is the "species" (Mace, 2004; Green, 2005). For many biota, the species is a clear and distinct biological grouping. It is superficially an easily understood unit, both politically and amongst the general public (Diamond, 1966) and it forms the basis of the vast majority of conservation legislation in the UK, Europe and elsewhere. For example, of the 8070 current taxon designations in the Global Red List, CITES, Bonn Convention, Berne Convention, EU Habitats and Species Directive, EU Wild Birds Directive, UK Wildlife and Countryside Act 1981 and UK Biodiversity Action Plan list, 98.2% are species designations, with the remainder at infra-species level (mostly sub-species but some plants are designated for hybrids). No

[1] This chapter has been submitted as a manuscript to *Advances in Limnology* as part of a special publication for the International Coregonid Symposium 2008.

mammals, fish, amphibians or reptiles are currently designated at an infra-species taxonomic level.

Despite the heavy reliance on the species as a conservation unit there are some theoretical and practical difficulties associated with its use as a pragmatic conservation tool. Firstly, there continues to be significant theoretical difficulty in achieving a conceptual consensus of exactly what defines a species (Mayden, 1997; Coyne & Orr, 2004). For many species, alternative species concepts deliver the same outcome, however for more "difficult" species, differing conceptual frameworks for species can result in significant practical consequences for legislation and policy (Kottelat, 1998; Agapow *et al*, 2004). Secondly, conservation of a whole species may not be realistic, especially if management intervention is required, given that resources are usually constrained. In this case conservation at a level lower than whole species might be more appropriate (DeGuia & Saitoh, 2007). Thirdly, for some species, there may be some significant structuring within the species that can have conservation importance. For many species, the population (defined as a group of randomly interbreeding individuals of the same species) and the species may be functionally identical. However for some species, populations may be quite distinct from each other; this can be significant for protection and conservation.

There have been a number of attempts to theoretically define units for conservation below that of species. Ryder (1986) proposed the concept of the "Evolutionarily Significant Unit" (ESU) as a population "*possessing genetic attributes significant for present and future generations*". This purely genetic definition has been modified elsewhere to also include discrete phenotypic characters and restricted gene flow (see DeGuia & Saitoh, 2007 for review of its use). Rader *et al.* (2005) suggest that one method to define an ESU is to estimate "ecological exchangeability" between populations. When populations demonstrate statistical differences in morphological, behavioural, life-history and genetic traits under selection, or occupy different habitats, ecological exchangeability would be rejected and populations would become important conservation units.

The conservation status of infra-specific units is recognised in legislation outwith Europe. The US Endangered Species Act 1978, for example, includes provision for listing "population segments". Similarly the Canadian Species at Risk Act 2002 and Australian Environment Protection and Biodiversity Conservation Act 1999, allow for the consideration of distinct 'populations' for listing. However, in the UK, neither the Wildlife and Countryside Act (1981), which in Scotland, is supplemented by the Nature

Conservation (Scotland) Act (2004), make specific provisions for infra-species level protection for vertebrates. The Global Red List does however, list a significant number of sub-species particularly for birds. However sub-specific taxonomic levels are not fully recognised in other international or European-level conservation legislation.

Habitats that promote infra-specific structuring are frequently fragmented; in Scotland postglacial freshwater systems represent a good example of such a habitat. European whitefish (*Coregonus lavaretus*), known as powan in Scotland, is a species that exhibits significant variation between populations to the extent that there has been much discussion about its taxonomic status (Kottelat & Freyhof, 2007). This species is of conservation interest in the UK due to the small number of populations present. In the UK, *C. lavaretus* is fragmented into seven discrete native lake-dwelling populations, one in Wales, four in north-west England and two in Scotland (Loch Lomond and Loch Eck). All UK populations inhabit postglacial systems thought to have been recolonised by sea routes from ca. 10,000 years ago (Maitland, 1970; Maitland, 1994).

The general aim of this paper is to determine if powan might benefit from management at infra-species population level in Scotland. To do this two key biological hypotheses are posed related to the nature of the two native populations in Scotland. If a common management strategy for both Lomond and Eck populations is to be effective, it would be expected that the following null hypotheses to hold:
1) There are no significant morphological differences between populations; and 2) There are no significant ecological differences between populations.
These are tested here.

3.3 METHODS

3.3.1 Sites

Loch Lomond and Loch Eck are located in different catchments in west central Scotland (for additional details see Appendix 1). They differ in size and bathymetry, but are only 22 km apart and their catchments are geologically and climatically similar. Loch Lomond (56°5'N, 4°36'W) has the largest surface area of any freshwater body in Scotland (ca. 71 km^2); it has a maximum depth of 190 m, and is 8 m above sea level. It has three basins, the north basin is deep and narrow, while the south basin is wide and shallow and these are divided by an intermediate mid basin (Tippett *et al*, 1974; Tippett, 1994). Loch Lomond supports a diverse native fish fauna and has also been colonised by a number of invasive fish species (Adams, 1994; Etheridge & Adams, 2008). Loch Eck (56°6'N, 4°59'W) has a

surface area of ca. 4.6km^2, has a maximum depth of 42 m, is 9m above sea level, and has a much simpler bathymetry than Loch Lomond. Loch Eck supports a less diverse fish fauna, all of which are native, but receives conservation protection because of the unusual mixture of species found there, which includes Arctic charr (*Salvelinus alpinus*).

3.3.2 Fish collection

Multi-panel Nordic-pattern gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the two lochs over the winter of 2005/06. Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986). In total, 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (from 9 November 2005 to 24 January 2006), six gill nets were set overnight in Loch Eck (from 9 January 2006 to 10 January 2006). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds. During this period a total of 341 powan were caught (Loch Lomond n = 118 and Loch Eck n = 223). Fish were frozen within four hours of capture (for additional details see Appendix 2).

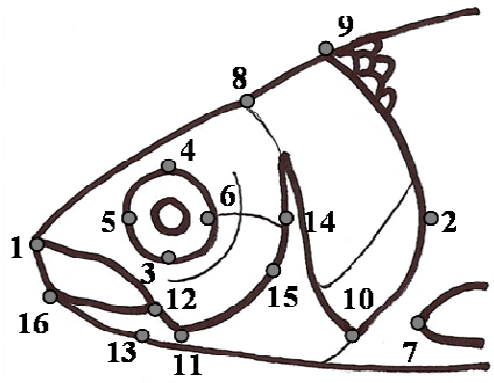
3.3.3 Catch data

Fish catch rate was calculated as CPUE (catch-per-unit-effort), determined as the number of powan caught per 12 hours per net. Nets set outwith the month when the most powan were caught (between 30/12/05 and 24/1/06) were excluded. Nets set at sites where no powan were ever caught were excluded. The CPUE was compared between lochs using ANOVA.

3.3.4 Head morphology

The fish were laid out on 2 mm graph paper. Digital photographs of the left side of the fish were taken using a Nikon Coolpix digital camera, attached to a copy stand with sufficient lighting. Thin Plate Spline (TPS) is a landmark-based geometric technique for the determination of shape. The method provides a powerful analysis of shape independent of size (Rohlf, 1990, 2002; Bookstein, 1991). For the analysis of morphology (morphometrics software was downloaded from http://life.bio.sunysb.edu/morph), images of 40 individuals from each site were chosen that gave good detail of the head. A total of 14 landmarks (Fig. 3.1.) were identified on the head and digitised to coordinates using the programme "TpsDig". Procrustes superimposition in the programme "Coordgen6", with landmarks 1 and 2 as baseline end-points, was used to rotate, translate and scale procrustes coordinates.

Based on the obtained procrustes coordinates, new shape variables, partial warp scores are calculated in the programme "PCAGen". These new variables capture spatial variation in a sample and can be used in statistical analysis. The partial warps were reduced by principal components analysis (PCA) and MANOVA was performed on the resulting component scores to test the effect of loch of origin.



Landmark	Description
1	Snout
2	Most posterior point of operculum
3 - 6	Eye
7	Base of pectoral fin
8, 12, 14	Anterior side of operculum; dorsal, mid and ventral points
9, 10	Posterior side of operculum; dorsal and ventral points
11	Edge of maxillary
13	Depth of lower jaw under maxillary

Figure 3.1 The 16 landmarks chosen to describe shape of the head of powan

3.3.5 Determination of size and age

Fork length (L_f) (to 1 mm) and weight (W) (to 0.01 g) were measured. Scales were removed from the flank below the dorsal fin for age determination. The clearest three scales were selected and impressed onto plastic. A projection microscope was then used to determine age, and the median age (determined from three scales) was taken as the age of the individual in order to minimise any impact of reading errors. The specimens were dissected and sex was determined by the visual examination of gonads. The outer surface of the stomach and gut was examined for cysts of the parasite *Diphyllobothrium spp.*, and scored for the number of cysts of each individual fish as follows: 0 = 0, 1 = 1-9, 2 = 10-19, 3 = 20-50, 4 = 50+. The adipose fin was removed and stored in 100% ethanol. Genetic work on this tissue took place in the Fisheries Research Services laboratory in Pitlochry and has been reported on by Thompson *et al.* (2008).

3.4 RESULTS

The catch rate of powan was significantly different between populations (ANOVA, $F_{1,38}$ = 78.7, $p < 0.001$) (Fig. 3.2.). The first three principal components (PC) of a PCA of partial warp scores derived from TPS analysis each explained more than 10 % of the variance

found in head shape. There was a significant difference between powan from Loch Lomond and Loch Eck across all three PCs (MANOVA, $F_{2,76} = 6.0$, $p < 0.001$). PC1 explained 11.4 % of the total variation in the partial warp scores. However, there was no significant difference in PC1 between the two populations (ANOVA, $F_{1,78} = 1.1$, $p = 0.31$). PC2 explained 11.0 % of the total variation in the partial warp scores and was significantly different between populations (ANOVA, $F_{1,78} = 8.1$, $p < 0.01$). A high PC2 score corresponds with a deeper head, the eye being more anterior and the snout and mouth shorter. A low PC2 score corresponds with a shallower head, the eye being more posterior and the snout and mouth longer (Fig. 3.3.). PC3 explained 10.4 % of the total variation in the partial warp scores and was significantly different between populations (ANOVA, $F_{1,78} = 7.1$, $p < 0.01$). A high PC3 score corresponds with the pectoral fin being in a more anterior and ventral position and the eye being more posterior and dorsal. A low PC3 score corresponds with the pectoral fin being more posterior and dorsal, and the eye being more anterior and slightly more ventral (Fig. 3.3.).

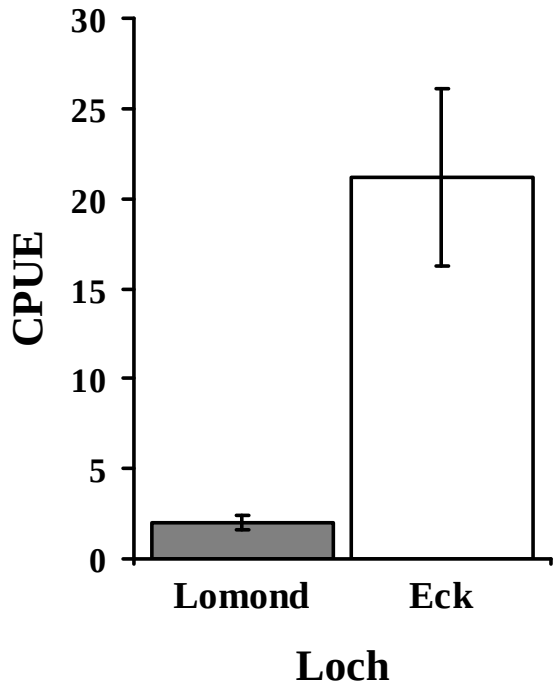


Figure 3.2 Mean (± S.E.) CPUE (powan caught per 12 hours per net) of powan from Loch Lomond (2.0 ± 0.41), and Loch Eck (21.1 ± 4.9).

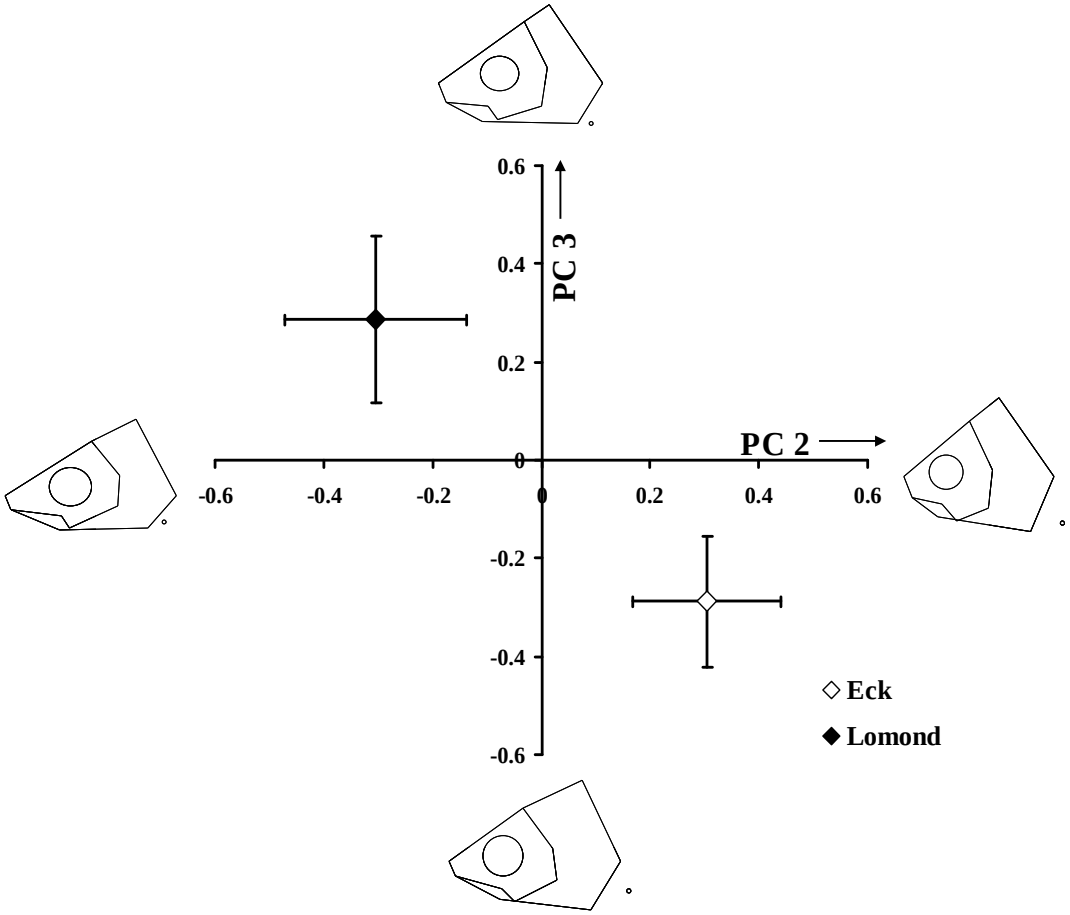


Figure 3.3 Mean (± S.E.) PC2 and PC3 describing the head of powan from Loch Lomond (PC2, -0.30 ± 0.17; PC3, 0.29 ± 0.17) and Loch Eck (PC2, 0.30 ± 0.14; PC3, -0.29 ± 0.13). Head shape extremes (exaggeration 1.5) are shown to illustrate landmark movement at high and low PC2 and PC3

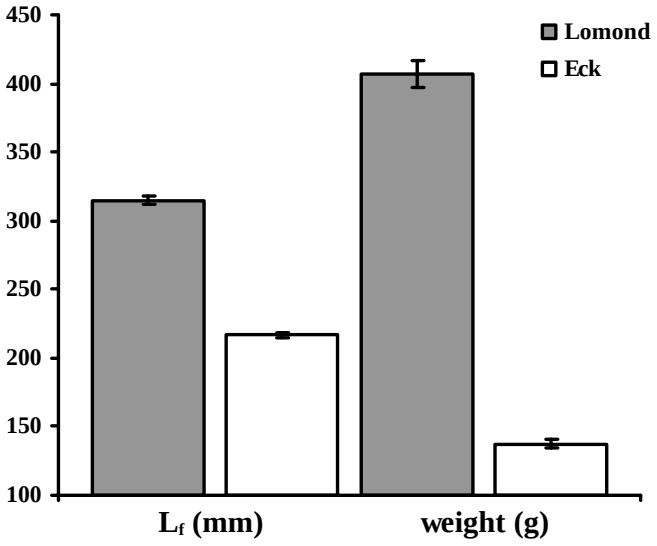


Figure 3.4 Mean (± S.E.) L_f and weight. Loch Lomond powan had a mean L_f 315mm ± 3.1. Loch Eck powan had a mean L_f 217mm ± 2.1. Loch Lomond powan had a mean weight 406.71g ± 9.8. Loch Eck powan had a mean weight 137.36g ± 3.3

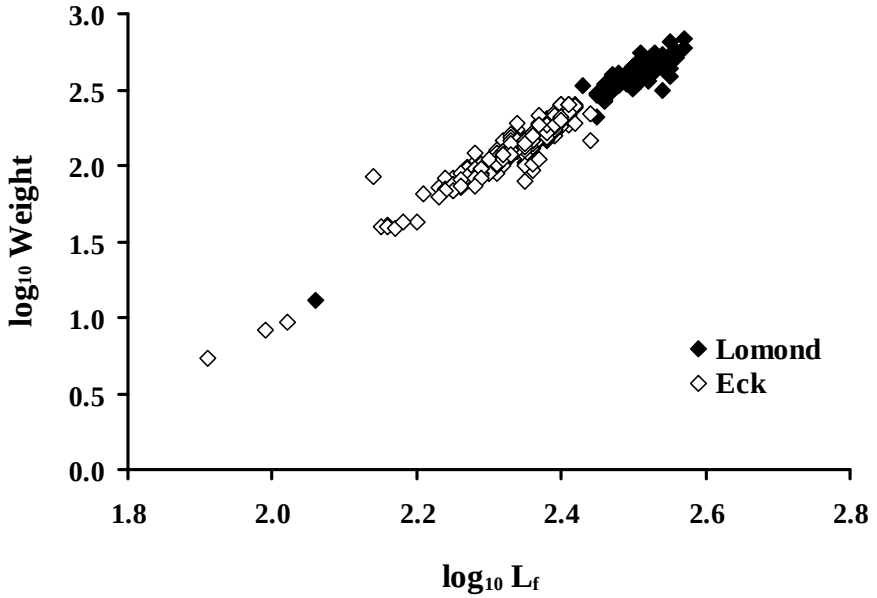


Figure 3.5 $log_{10}L_f$ and log_{10}Weight of powan from Loch Lomond and Loch Eck. There is a significant linear correlation between $log_{10}L_f$ and log_{10}Weight for each site: Loch Lomond (y = 3.1x – 5.1) and Loch Eck (y = 2.7x – 4.2).

The difference in L_f (ANOVA, $F_{1,346} = 740.1$, $p < 0.0001$) and weight (ANOVA, $F_{1,346} = 975.0$, $p < 0.0001$) between Loch Lomond and Loch Eck powan is significant. There was a significant linear correlation between $log_{10}L_f$ and log_{10}Weight for each site: Loch Lomond ($R^2 = 0.91$; ANOVA, $F_{1,123} = 1305.9$, $p < 0.0001$); and Loch Eck ($R^2 = 0.92$; ANOVA, $F_{1,221} = 2396.1$, $p < 0.0001$). Loch Lomond powan were longer and heavier than Loch Eck powan (Fig. 3.4.). However, the relationship between and log_{10}Weight and $log_{10}L_f$ is not significantly different (ANCOVA, $F_{1,345} = 0.52$, $p = 0.47$) between sites (Fig. 3.5.).

There was no significant difference between the mean age of powan from Loch Lomond and Loch Eck (ANOVA, $F_{1,342} = 3.2$, $p = 0.07$). L_f at age of powan from Loch Lomond and Loch Eck were best described by quadratic relationships: Loch Lomond ($R^2 = 0.77$; ANOVA, $F_{2,120} = 199.1$, $p < 0.0001$) and Loch Eck ($R^2 = 0.81$; ANOVA, $F_{2,218} = 452.5$, $p < 0.0001$) (Fig. 3.6.). These differed significantly (F-test, $F_{2,341} = 1011.1$, $p < 0.0001$), with Loch Lomond powan having the greatest L_f at all ages.

Loch Lomond powan had a significantly higher parasitic load of *Diphyllobothrium spp.* than those from Loch Eck (Mann-Whitney U, $p < 0.0001$) (Fig. 3.7.). There was also a significant difference between the frequency of infected powan between Loch Lomond

(infection occurrence 99 %) and Loch Eck (infection occurrence 89 %) ($\chi^2 = 11.3$, d.f. = 1, $p < 0.01$).

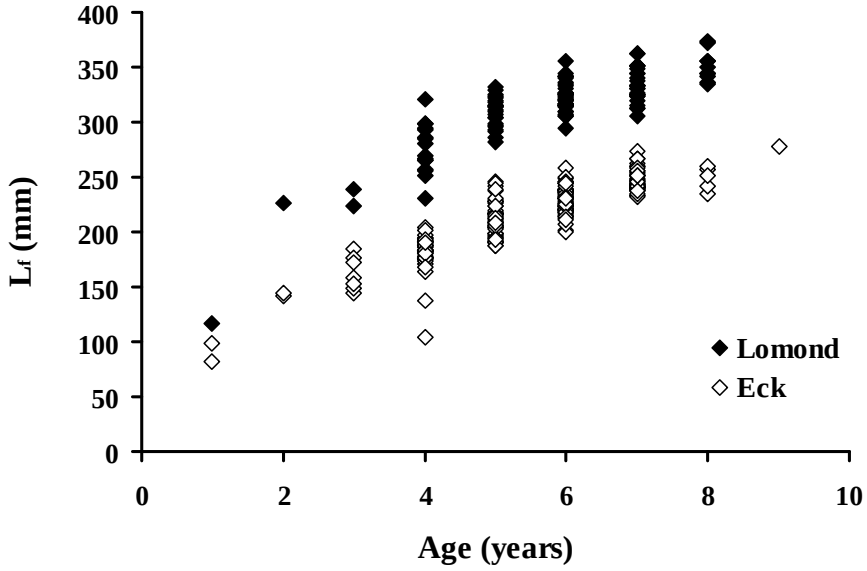


Figure 3.6 L_f at age of powan from Loch Lomond and Loch Eck. There is a quadratic relationship between age and L_f for both populations: Loch Lomond ($y = 65.8x - 4.1x^2 + 78.0$); and Loch Eck ($y = 39.8x - 1.8x^2 + 55.0$).

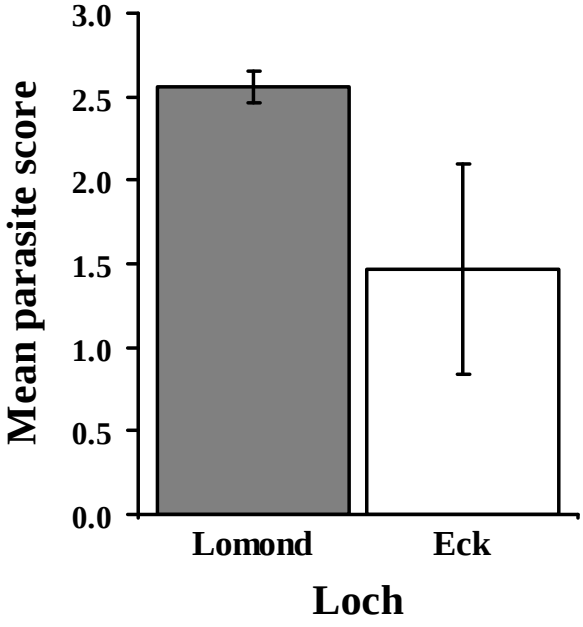


Figure 3.7 Parasite scores (mean ± S.E.) of powan from Loch Lomond (2.6 ± 0.10), and Loch Eck (1.5 ± 0.06). Score: 0 = 0, 1 = 1-9, 2 = 10-19, 3 = 20 – 49, 4 = 50+.

3.5 DISCUSSION

Isolated populations of the same species are potentially exposed to differing evolutionary pressures, which can result in between-population infra-species divergence. If such evolutionary pressures result in significant between-population divergence in phenotype, ecology or behaviour, it is likely that different populations may require different management strategies formulated on population-specific characteristics. Here two hypotheses designed to examine the degree to which there is infra-specific structuring within powan in Scotland are tested.

The null hypothesis that there are no significant morphological differences between powan populations in Scotland is rejected. Here it has been shown that there is a significant difference in the head shape between powan of the two populations. Compared with Loch Eck powan, Loch Lomond powan had a shallower head with a longer snout and mouth, the mouth placed more dorsally, the eye placed more posteriorly and dorsally, and the pectoral fin closer to the head. While in comparison to Loch Lomond powan, Loch Eck powan had a deeper head with a shorter snout and mouth, the mouth placed more ventrally, the eye placed anteriorly and more ventrally, the pectoral fin placed more posteriorly. The shape of the head has a high level of functional significance being critical in the detection, capture and consumption of prey items. Head shape has been shown to correlate strongly with diet (Gardner *et al*, 1988; Adams & Huntingford, 2002; Wintzer & Motta, 2005).

The null hypothesis that there are no significant ecological differences between powan populations in Scotland is also rejected. Here I have shown that powan in Loch Eck are smaller, have a lower growth rate, and a lower *Diphyllobothrium spp.* parasite load compared with powan from Loch Lomond. These differences are consistent with earlier studies (Brown & Scott, 1990; 1994) which showed body size and growth differences and with those of Dolezel & Crompton (2000) which showed different parasitic loads between populations. Thus many of the ecological differences reported here are persistent characteristic differences between the populations (see also Pomeroy, 1991).

Literature on powan ecology is population-biased with no studies focusing solely on Loch Eck powan. Almost all studies on this species in Scotland have concentrated on the Loch Lomond powan (e.g. Gervers, 1954; Slack, 1955; Maitland, 1969; Fuller *et al*, 1974, 1976; Brown *et al*, 1991; Dorucu, 2000), or have compared only Loch Lomond powan with other populations of whitefish outside Scotland (e.g. Dottrens, 1959; Bridges & Yoshikami,

1970; Ferguson, 1974; Ferguson *et al*, 1978). However, the few comparative studies between Scottish powan which are available have shown additional disparities exist between these populations. Differences identified previously in powan of these two populations include: age at maturity, timing of spawning (Brown & Scott, 1994), gonodosomatic indexes, somatic condition factors (Brown & Scott, 1990) and more planktonic/benthic feeding behaviour (Pomeroy, 1991). Dolezel & Crompton (2000) suggested different levels of parasite infestations in powan in Loch Lomond and Loch Eck could be a reflection of their different feeding patterns, since this infection is attained through feeding on zooplankton. Although there is overlap in diet, and the infection rates of the planktons are not known, previous studies have found that Loch Lomond powan feed primarily on plankton, while Loch Eck powan feed primarily on benthos (Brown & Scott, 1990; Pomeroy, 1991), though sometimes on plankton (Slack *et al*, 1957). Furthermore, recent genetic work by Thompson *et al* (2008) on UK coregonids using microsatellites and mtDNA describes the existence of significant genetic differences between these powan from Loch Lomond and Loch Eck.

Additional dissimilarities between these populations are indicated by the CPUE of powan and the catch composition of these nettings, which further highlights the need to differentiate management plans. The low CPUE of powan in Loch Lomond in comparison to Loch Eck suggests that the heavier utilisation and the introduction of invasive species, in particular ruffe (*Gymnocephalus cernuus*) a known predator of powan eggs (Sterligova & Pavlovskiy, 1985; Adams & Tippet, 1991) and the most numerous fish in the Loch Lomond net catches (Appendix 2), has taken its toll at this site. Powan have previously been described as being the most numerous fish in Loch Lomond by Slack *et al* (1957) and as ‘very numerous’ by Brown & Scott (1994). These data therefore suggest that there has been a recent decline in the numbers of Loch Lomond powan; this has also been confirmed by recent hydroacoustic population work at both of these sites (Winfield *et al*, 2005). The catch compositions of the net catches at these sites highlight another reason for separate management strategies, in the very different fish communities. Loch Eck is one of only two populations in the UK where *Coregonus* and Arctic charr coexist; while Loch Lomond is species-rich and is home to an unusual freshwater-resident morph of the river lamprey (*Lampetra fluviatilis*) that appears to specialise on parasitizing powan (Maitland, 1980; Adams *et al*, 2008).

It is concluded that there is very strong evidence that these populations do not represent ‘ecologically exchangeable’ units. There are a number of consequences that result from

this conclusion. Firstly powan from Loch Lomond and Loch Eck should be considered as separate ESUs, with different management strategies. Secondly the structuring within Scottish powan should be recognised in the national conservation designation framework. A fish conservation framework such as this, would almost certainly be included within a Red Data Book for UK fish, and it is a matter of concern that such an assessment has yet to be carried out. However, some provision already exists for the protection of some species of freshwater fish at the infra-specific level. The UK Biodiversity Action Plan (UK BAP) list guidance for example, advises that: "*coordinators should help species specialist groups decide the taxonomic level at which they will work (sub-species, race variety etc.)*" (www.ukbap.org.uk/library/brig/shrw/TerrFwSppGuidance.pdf). Similarly, the guidelines for the selection of Biological Sites of Special Scientific Interest (SSSIs) clearly state that "*ecotypic or genetically distinctive fish populations…..are worthy of conservation*". Although powan is focussed on here, it is suggested that a significant number of other species may show infra-specific structuring of phenotype and/or genotype such that conservation management strategies designed at the species level are highly inappropriate. It is suggested that such species are most likely to be found in post-glacial freshwater systems in species with limited powers of intersystem dispersal.

Chapter 4 MORPHOLOGICAL AND ECOLOGICAL RESPONSES TO A CONSERVATION TRANSLOCATION OF POWAN (*COREGONUS LAVARETUS*) IN SCOTLAND[2].

4.1 ABSTRACT

The establishment of refuge populations has become a common management tool for threatened fish species in recent years, yet the effects of translocation are not fully understood in a conservation context. Here I test the hypothesis that phenotypic changes have occurred during the formation of two refuge populations of the nationally rare powan (a freshwater fish species) which were established in Loch Sloy and Carron Valley Reservoir. Significant morphological and ecological differences between the source (Loch Lomond) and refuge populations and between the two refuge populations were demonstrated. These changes are probably due to a combination of founder effects, intense selection and phenotypic plasticity. These changes can undermine the rationale behind the establishment of refuge populations. The results call into question the usefulness of translocation as a conservation measure, however, there are times when this is the only viable management option available. The future of translocation and the validity of establishing refuge populations for powan conservation are discussed.

4.2 INTRODUCTION

Despite its extensive use as a tool for conservation and management in both plants (Maunder, 1992; Jusaitis & Polomka, 2008) and animals (McGrady *et al.*, 1994; Hauser *et al.*, 1995; Denton, 1997; Yamamoto *et al.*, 2006; Hochkirch *et al.*, 2007; Kinley & Newhouse, 2008), the biological implications of translocation in a conservation context remain poorly understood (Stockwell *et al.*, 1996). Nevertheless, translocation is likely to become more commonly used as a conservation tool as existing populations are threatened in response to impacts such as climate change, or as formerly degraded habitats are restored (Hendrickson & Brooks, 1991; Maitland & Lyle, 1992).

Translocation has often been used to supplement existing populations either to enhance gene flow between populations (Yamamoto *et al.*, 2006), or to increase numbers in the recipient population (Weeder *et al.*, 2005). However, translocations into existing populations of the same species may encounter problems when local populations are genetically distinct or locally adapted (Gharrett & Smoker, 1991; Leary *et al.*. 1995).

[2] This chapter has been accepted as a paper in *Aquatic Conservation: Marine and Freshwater Ecosystems* (Etheridge *et al.*, in press).

Translocation may also be used to form new populations, either to re-establish the species where it has become locally extinct, or to form refuge populations in order to form a numerical buffer to extinction (Hendrickson & Brooks, 1991; Maitland & Lyle, 1992). However, there are good theoretical reasons to suggest that new populations established using translocation as a tool may not exactly replicate the features of the donor population. The movement of effectively small numbers of individuals has the potential to restrict genetic variability in the establishing populations (Nei *et al.*, 1975; Stockwell *et al.*, 1996). In addition the new environment to which the new population is exposed could potentially shape the expression of local adaptations not found in the originating population (Robinson & Schluter, 2000).

In practice, because of the complexity of the various processes involved and the potentially subtle interactions with a new environment, it is difficult to predict the eventual outcome of these processes for any translocation (Maitland *et al.*, 1991; Robinson & Wilson, 1994; Kirchhofer, 1995; Raitaniemi *et al.*, 1999). Whatever the main drivers may be, the potential for changes in a translocated species at both an individual and a population level may have significant implications for conservation of the species concerned. Significant phenotypic and genetic changes in fish populations following translocation are relatively frequently reported in the literature (Loch. 1974; Vuorinen *et al.*, 1991; Shields & Underhill, 1993; Hauser *et al.*, 1995; Quinn *et al.*, 2001; Weeder *et al.*, 2005; Lema, & Nevitt, 2006) (but see DeMarais & Minckley, 1993).

The powan (*Coregonus lavaretus*) occurs naturally in only seven lakes within the UK. Two of these populations occur in Scotland, in Loch Lomond and Loch Eck, and are known to differ in some aspects of their feeding strategies, growth and metabolism (Brown & Scott, 1990; 1994). Loch Lomond supports the largest population of powan in Scotland, however, due to a variety of catchment based anthropogenic pressures and in particular the introduction of many invasive non-native species (Adams & Tippett, 1991; Etheridge & Adams, 2008), fish from the Loch Lomond population were translocated to form two refuge populations in Loch Sloy and Carron Valley Reservoir Powan between 1988 and 1991 (Maitland & Lyle, 1992; 1995). 12,227 fry and 85 adults were introduced into Loch Sloy and 13,123 fry were introduced into Carron Valley Reservoir (Maitland & Lyle, 1991b). The aim of this paper is to determine whether the establishment of refuge populations of Lomond powan has resulted in a phenotypic change and to critically examine the impacts that any observed changes may have on the validity of using refuge populations as a tool for conserving rare endangered populations of freshwater fish.

4.3 METHODS

4.3.1 Sites

The three study sites are situated in west central Scotland (Appendix 1). They differ in size and bathymetry, but are located within the same catchment. Loch Lomond (56°5'N, 004°36'W) has the largest surface area body of fresh water in Scotland (ca. 71 km^2); it has a maximum depth of 190 m, and is 8 m above sea level. It has three basins, the north basin is deep and narrow, while the south basin is wide and shallow and these are divided by an intermediate mid basin (Tippett *et al.*, 1974). Loch Lomond hosts a variety of native and recently introduced non-native species (Etheridge & Adams, 2008). Powan are an integral part of the native community and this is demonstrated by its ecological relationship with a unique non-sea migrating population of river lamprey (Adams *et al.*, 2008). Loch Sloy (56°16'N, 004°47'W) is a small semi-natural loch to the north-west of Loch Lomond, it has a surface area of ca. 1 km^2, a maximum depth of ca. 40 m, and is 287 m above sea level (Tippett *et al.*, 1974). It is divided into a small shallow north basin and a larger and deeper south basin (Murray & Pullar, 1910). The site is located at high altitude and is shaded by surrounding mountains, surface water temperatures are therefore lower throughout the year than those observed in either of the other sites. This site appears to host only small brown trout (*Salmo trutta*) and predation risk for adult powan is considered to be absent. The water supply of Loch Sloy is used to generate hydroelectric power and as a consequence to this, the loch margins and potential spawning areas can be exposed during periods of peak usage. Carron Valley Reservoir (56°2'N, 004°6'W) is located to the east of Loch Lomond. It has a surface area of ca. 3 km^2, a maximum depth of ca. 10 m, and is 223 m above sea level (Tippett *et al.*, 1974). As a functional reservoir, the site is subject to water level fluctuations but potential spawning substrates are rarely exposed. The site has been utilised as a recreational put-and-take fishery for several decades and has been regularly stocked with brown trout, and in recent years rainbow trout (*Oncorhynchus mykiss*).

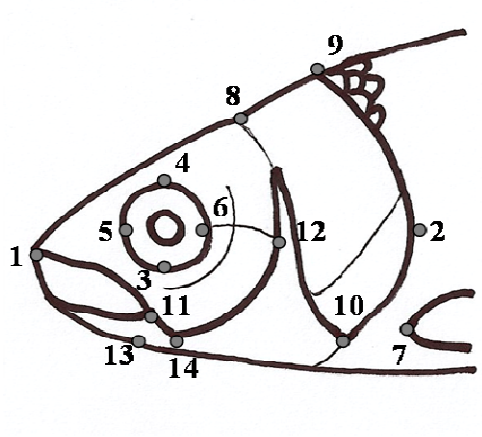
4.3.2 Fish collection

Multi-panel Nordic-pattern gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the three lochs over the winter of 2005/06. Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986). In total, 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (between 9 November 2005 to 24 January 2006), seven gill nets,

were set overnight in Loch Sloy (from 21 December 2005 to 28 December 2005) and ten gill nets were set overnight in Carron Valley Reservoir (from 3 January 2006 to 5 January 2006). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds. During this period a total of 252 powan were caught (Loch Lomond n = 118, Loch Sloy n = 76 and Carron Valley Reservoir n = 58). Fish were frozen within four hours of capture.

4.3.3 Catch data

Fish catch rate was calculated as CPUE (catch-per-unit-effort), determined as the number of powan caught per 12 hours per net. Nets set out-with the month when the most powan were caught (between 28/12/05 and 24/1/06) were excluded. Nets set at sites where no powan were ever caught were excluded. The CPUE was compared between lochs using ANOVA.



Landmark	Description
1	Snout
2	Most posterior point of operculum
3 - 6	Eye
7	Base of pectoral fin
8, 12, 14	Anterior side of operculum; dorsal, mid and ventral points
9, 10	Posterior side of operculum; dorsal and ventral points
11	Edge of maxillary
13	Depth of lower jaw under maxillary

Figure 4.1 14 landmarks chosen to describe shape of the head of powan

4.3.4 Head morphology

The fish were laid out on 2 mm graph paper. Digital photographs of the left side of the fish were taken using a Nikon Coolpix digital camera, attached to a copy stand with sufficient lighting. Thin plate spline (TPS) is a landmark-based geometric technique for the determination of shape (morphometrics software downloaded from http://life.bio.sunysb.edu/morph). The method provides a powerful analysis of shape independent of size (Rohlf, 1990; Bookstein, 1991). For the analysis of morphology, images of 40 individuals from each site were chosen that gave good detail of the head. A total of 14 landmarks (Fig. 4.1.) were identified on the head and digitised to coordinates using the programme "TpsDig". Procrustes superimposition in the programme

"Coordgen6", with landmarks 1 and 2 as baseline end-points, was used to rotate, translate and scale landmarks and produce procrustes coordinates. Based on the obtained procrustes coordinates, new shape variables, partial warp scores were calculated in the programme "PCAGen". These new variables capture spatial variation in a sample and can be used in statistical analysis. The partial warps were reduced by Principal Components Analysis (PCA) and an ANOVA was performed on the resulting component scores to test the effect of loch of origin.

4.3.5 Life History

Fork length (L_f) (to 1 mm) and weight (W) (to 0.01 g) were measured. Scales were removed from the flank below the dorsal fin for age determination. The clearest three scales were selected and impressed onto plastic. A projection microscope was then used to determine age, and the median age (determined from three scales) was taken as the age of the individual in order to minimise any impact of reading errors. The specimens were dissected and sex was determined by the visual examination of gonads. The outer surface of the stomach and gut was examined for cysts of the parasite *Diphyllobothrium spp.*, and scored for the number of cysts of each individual fish as follows: 0 = 0, 1 = 1-9, 2 = 10-19, 3 = 20-50, 4 = 50+. The adipose fin was removed and stored in 100 % ethanol. Genetic work on this tissue has been reported on by Thompson *et al.* (2008).

4.4 RESULTS

4.4.1 Catch rate

The catch rate of powan was significantly different between sites (ANOVA, $F_{2,52} = 12.8$, $p < 0.0001$). Bonferroni *post hoc* testing showed that the CPUE was significantly lower in Loch Lomond compared with Loch Sloy ($p < 0.0001$) and lower in Carron Valley Reservoir than in Loch Sloy ($p < 0.01$), but that was no significant difference in the CPUE between Loch Lomond and Carron Valley Reservoir ($p = 0.30$) (Fig. 4.2.).

4.4.2 Head morphology

PC1 explained 16.6 % of the total variation in landmark position. Fish with an extreme positive PC1 score were typified by a deeper head, a more anterior eye and a reduced snout and mouth, with most landmarks being more dorsally placed, compared with an extreme negative PC1 score. Differences in PC1 scores between the three populations were significant (ANOVA, $F_{2,117} = 9.8$, $p < 0.001$). Bonferroni post hoc testing showed a significant difference between Loch Lomond and Loch Sloy ($p < 0.05$) and between Loch

Sloy and Carron Valley Reservoir ($p < 0.0001$), but there was no significant difference between Loch Lomond and Carron Valley Reservoir ($p = 0.37$).

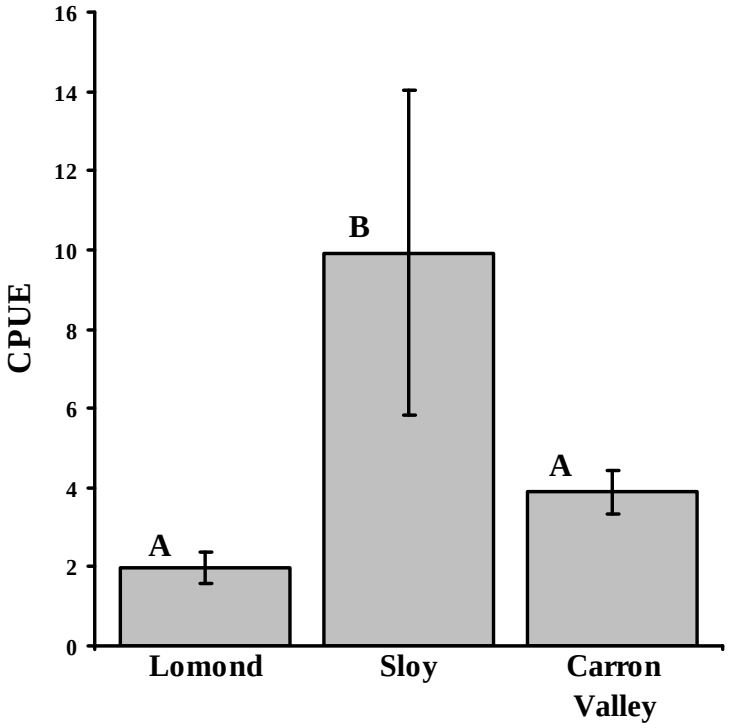


Figure 4.2 Mean (± S.E.) catch rate of powan per 12 hours of netting per 30 m Nordic gill net, from Loch Lomond, Loch Sloy and Carron Valley Reservoir. Post-hoc pairwise comparisons: similar alphanumeric character = no significant difference, other differences at $p < 0.01$

PC2 explained 13.9 % of the variance seen in landmark position. Fish with an extreme positive PC2 score were typified by a longer and shallower head, a more ventrally placed pectoral fin, with most landmarks being more posteriorly placed, compared with an extreme negative PC2 score. Differences in PC2 scores between the three populations were significant (ANOVA, $F_{2,117} = 15.2$, $p < 0.0001$). Bonferroni *post hoc* testing showed a significant difference between Loch Lomond and Carron Valley Reservoir ($p < 0.0001$) and between Loch Sloy and Carron Valley Reservoir ($p < 0.0001$), but there was no significant difference between Loch Lomond and Loch Sloy ($p = 0.99$) for PC2 scores.

Compared with Loch Lomond powan, Loch Sloy powan had a shallower head, a more posterior eye, a longer snout, a more dorsally placed pectoral fin and a longer mouth. Compared with Loch Lomond powan, Carron Valley Reservoir powan had a shallower head, a more posterior eye, a longer snout and a more anteriorly placed pectoral fin. While,

compared with Loch Sloy powan, Carron Valley Reservoir powan had a deeper head, a more anterior and dorsal eye, a shorter snout, a more posterior and ventral pectoral fin, a shorter mouth with a more ventral mouth edge (Fig. 4.3.).

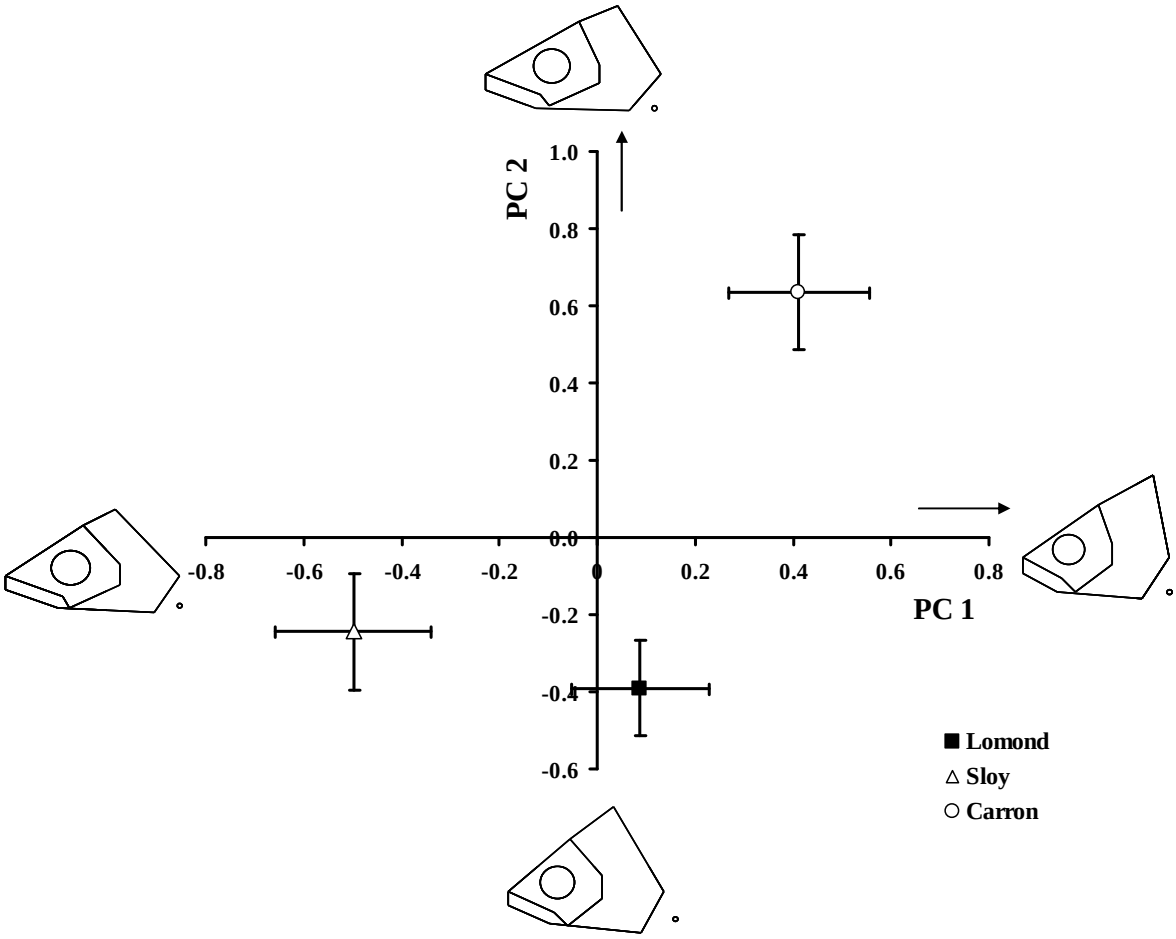


Figure 4.3 Mean (± S.E.) PC1 and PC2 scores describing the head of powan from Loch Lomond, Loch Sloy and Carron Valley Reservoir

4.4.3 Life history

There was a significant difference in fork length (L_f) (ANOVA, $F_{2,255} = 123.2$, $p < 0.0001$) and weight (ANOVA, $F_{2,255} = 195.0$, $p < 0.0001$) between sites. Bonferroni *post hoc* testing showed that all between population, pairwise comparisons of L_f and of weight were significantly different ($p < 0.0001$). Log_{10} weight (g) at log_{10} L_f (mm) of powan from Loch Lomond ($F_{1,123} = 1318.8$, $R^2 = 0.92$, $p < 0.0001$), Loch Sloy ($F_{1,74} = 69.3$, $R^2 = 0.48$, $p < 0.0001$) and Carron Valley Reservoir ($F_{1,56} = 904.7$, $R^2 = 0.94$, $p < 0.0001$) is best described with a linear model. Carron Valley Reservoir powan were on average the largest (length and weight) of the three sites, Loch Sloy powan the smallest, and Loch Lomond powan intermediate (Fig. 4.4.). The increase of log_{10}weight with $log_{10}L_f$ was lowest for

Loch Sloy powan, the slope of which is significantly different from that of Loch Lomond powan (ANCOVA, $F_{1,198} = 8.9$, $p < 0.01$) and from that of Carron Valley Reservoir powan (ANCOVA, $F_{1,131} = 43.7$, $p < 0.0001$). There was no significant difference in the length weight relationship between Loch Lomond and Carron Valley Reservoir powan (ANCOVA, $F_{1,180} = 3.3$, $p = 0.57$) (Fig. 4.4.).

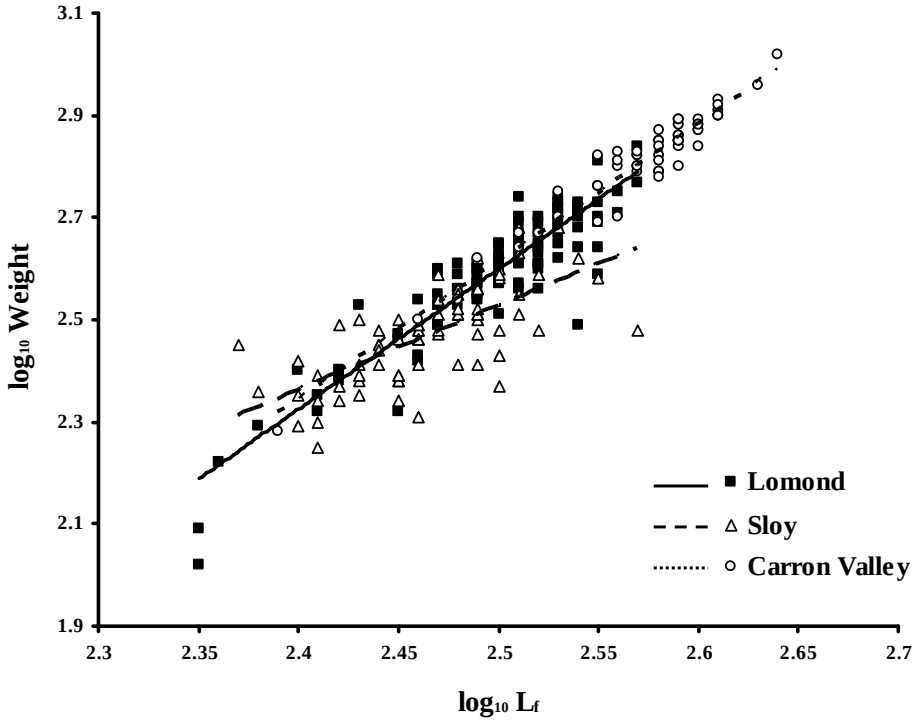


Figure 4.4 Log_{10} weight (g) at log_{10} L_f (mm) of powan from Loch Lomond (y = 3.1x – 5.1), Loch Sloy (y = 1.6x – 1.6) and Carron Valley Reservoir (y = 2.7x – 4.1) was best described with a linear model

The most frequent age in catches from all populations was 6+ years, however Loch Sloy powan had a significantly greater mean age (ANOVA, $F_{2,251} = 4.6$, $p < 0.05$) than Loch Lomond and Carron Valley Reservoir powan. Length (L_f) at age of powan from Loch Lomond ($F_{1,120} = 199.1$, $R^2 = 0.77$, $p < 0.0001$), Loch Sloy ($F_{2,73} = 15.9$, $R^2 = 0.30$, $p < 0.0001$) and Carron Valley Reservoir ($F_{2,53} = 111.8$, $R^2 = 0.81$, $p < 0.0001$) was best described with a quadratic model (Fig. 4.5.). The resulting curves are significantly different ($F_{2,251} = 124.6$, $p < 0.0001$). Carron Valley Reservoir powan had the greatest L_f at age, Loch Sloy powan had the lowest L_f at age, while Loch Lomond powan had an intermediate L_f at age.

There was a significant difference in the parasitic score between Loch Lomond powan and those from Loch Sloy (Mann-Whitney U, $p < 0.0001$), between Loch Lomond powan and Carron Valley Reservoir powan (Mann-Whitney U, $p < 0.0001$) and between Loch Sloy and Carron Valley Reservoir powan (Mann-Whitney U, $p < 0.0001$). Loch Lomond powan on average had the greatest parasite score (2.6 ± 0.1 S.E.), Carron Valley Reservoir powan had the lowest (0.65 ± 0.14 S.E.), while Loch Sloy powan had an intermediate score (1.3 ± 0.14 S.E.). Additionally there was a significant difference between the frequency of infected powan between Loch Lomond (infection occurrence 99 %), Loch Sloy (infection occurrence 75%) and Carron Valley Reservoir (infection occurrence 44 %) ($\chi^2 = 70.0$, d.f. = 2, $p < 0.0001$).

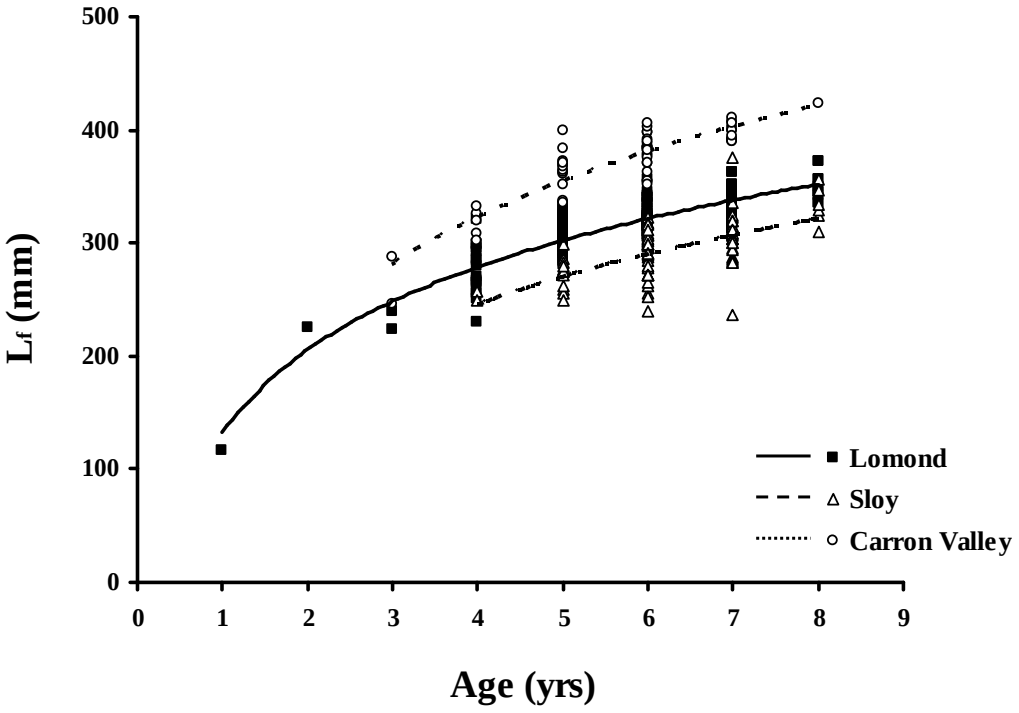


Figure 4.5 L_f at age of powan from Loch Lomond ($y = 65.8x – 4.1x^2 + 78.0$), Loch Sloy ($y = -4.6x + 1.9x^2 + 248.2$) and Carron Valley Reservoir ($y = 85.6x – 5.4x^2 + 64.0$) is best described with a quadratic model

4.5 DISCUSSION

In order to fulfil the function of a conservation refuge population, it is generally accepted that individuals from the refuge populations should not experience reduced survival if introduced back into the original site, that individuals should retain the potential for

breeding with the source population and that they should preserve the potential phenotypic and genotypic diversity found within the source population (Stockwell *et al.*, 1996).

Here it is shown that there are significant differences in the expressed phenotype between the powan of Loch Lomond and its conservation refuge population in Loch Sloy. Powan from Loch Sloy were smaller, had a lower weight for a given length, had a lower growth rate, a greater mean age and had a lower *Diphyllobothrium spp.* parasite load compared with Loch Lomond powan. This study has also shown significant differences in expression of phenotype between the powan of Loch Lomond and its conservation refuge population in Carron Valley Reservoir. Powan from Carron Valley Reservoir were larger, had a higher growth rate and a lower *Diphyllobothrium spp.* parasite load compared with Loch Lomond powan. Significant differences in expressed phenotype also differed between the two powan conservation refuge populations examined here. Powan from Carron Valley Reservoir were larger, had a greater weight for a given length, a higher growth rate, a lower mean age and a lower *Diphyllobothrium spp.* parasite load compared with Loch Sloy powan.

There are four possible mechanisms which could have resulted in the differences between refuge populations and donor population described here. Firstly, differences may be the result of a founder effect, where only a limited amount of the potential genetic variation was transferred to the refuge populations. Secondly, genetic drift, the loss of rare, non-selective alleles through random fluctuation of allele frequencies between generations may have occurred. Thirdly, there could be different selection pressures in the new environments compared to the donor environment. If this occurred, then different suites of local selection pressures could eventually lead to significant between population divergence. Finally phenotypic plasticity, the ability of a single genome to express multiple phenotypes, may have modified the expression of traits examined here. It seems unlikely that any one mechanism would be solely responsible for the sum of changes found between these populations; however, some factors may be less likely to have an influence than others.

Evidence of founder effects after translocation of fish species has been described (Hauser *et al.*, 1995; Quinn *et al.*, 2001; Weeder *et al.*, 2005). However, in the populations used in this study this effect is less likely. Fertilised eggs from powan from a mixed batch of Loch Lomond fish were used to establish both the Loch Sloy and Carron Valley Reservoir populations. Thus the initial genetic diversity at both populations was probably quite

similar and as a consequence, founder effects cannot provide a convincing explanation of the differences observed between the two refuge populations. Genetic drift is a non-selective process, therefore it is unlikely that a sufficient number of generations (ca. 4 generations, calculated from Brown *et al.*, 1991) has passed to allow genetic drift to occur. Using six microsatellites, Thompson *et al.* (2008) found mean allelic richness as a measure of genetic diversity to be 4.8 in Loch Lomond, 3.9 in Loch Sloy, and 4.2 in Carron Valley Reservoir, thus suggesting that there has been a very small loss of genetic diversity in the translocated populations. However, this loss after both the initial translocation and several generations in the new environment does not appear to be as great as might be if founder effects or genetic drift were the mechanisms causing the majority of phenotypic differences found between the populations in this study.

Genetic changes in a translocated fish population attributable to selection have previously been demonstrated (Vuorinen *et al.*, 1991). There is scope for selection as the cause of differences between populations. Head shape has a high level of functional significance and is critical in the detection, capture and consumption of prey items. It has been shown to correlate strongly with diet (Gardner *et al.*, 1988; Adams & Huntingford, 2002). Ecological and life history factors are likely to have several non-exclusive causes and reflect a series of tradeoffs from competing traits. Moreover, each of the sites varies in size, complexity, depth, altitude, shading, water level fluctuations and community structure in comparison to each other. It has been established (Thompson *et al.*, 2008), that there are significant detectable genetic differences between powan from Loch Lomond and Loch Sloy ($p < 0.005$), Loch Lomond and Carron Valley Reservoir ($p < 0.0001$) and between Loch Sloy and Carron Valley Reservoir powan ($p < 0.0001$), but this study used non-selected microsatellite markers and cannot be used as evidence of selection. Lastly, plastic effects following translocation of fish species have often been described (Loch, 1974; Shields & Underhill, 1993; Lema & Nevitt, 2006). Phenotypic plasticity cannot be ruled out as a major mechanism causing the differences between the populations in this study. Some traits shown to vary are known to be highly plastic (growth, maximum size, maturation size). The individuals that survive after translocation may be the ones most able to adapt quickly to a new environment and it is possible that newly founded populations might be ‘hyper-plastic’ (Parsons & Robinson, 2006). Selection and phenotypic plasticity are therefore likely to be the main causes of the differences observed between these populations in this study.

Additional dissimilarities between these populations are indicated by the CPUE. The possibility of a decline in Loch Lomond powan has previously been suggested. The low CPUE in Carron Valley Reservoir may be due to fewer individuals being present, alternatively this may have been caused by only the largest mesh sizes being capable of catching powan or this could simply be due to the netting in the refuge populations which was not carried out on or around historical spawning grounds and may not have hit spawning grounds successfully in Carron Valley Reservoir. The low parasite load in the refuge populations in comparison to that found in Loch Lomond suggests that the translocation may have given some release from *Diphyllobothrium spp.* infection. The higher incidence of infection in Loch Sloy may be explained by the fact that, in contrast to Carron Valley Reservoir, some adults were introduced alongside the newly hatched fry.

The results of this study raise questions about the value of conservation translocations. Powan in translocated populations in this study are different from the donor population in morphology, ecology, and life history. These differences are likely to be mainly due to phenotypic plasticity considering the speed of these changes, if this is found to be the case the conservation aims will not be undermined. However, if after a conservation translocation differential pressures persist over time they could result in significant between population divergences and thus have the potential to erode the initial rationale behind the creation of a refuge population.

These findings do not indicate that refuge populations and conservation translocations have no place in the management of threatened species. They merely indicate that the consequences of translocation for some species must be acknowledged prior to this type of management option taking place. Firstly, some species are known to be plastic and the phenotype may respond quickly to differential pressure. Secondly, if different environments are presented, some species show a significant genetic response to the different selection pressures present. It is in reality impossible to provide an exact replica of the source environment; therefore, the same plastic or genetic response will not be replicated in different environments. This response is likely to be species- and even population-specific, the actual impact that these factors may have will be hard to predict, but may be anticipated and possibly mitigated. There are certain actions that can be taken to minimise changes between translocated and donor populations. Effects of a small founding population such as founder, bottlenecking and genetic drift, can be avoided by introducing a suitably effectively large number of individuals over time, with associated monitoring (Hendrickson & Brooks, 1991; Stockwell *et al.* 1996). A translocation site can

be chosen that as closely as possibly resembles the donor site in physical and biotic factors. The effects of differential selection may then be minimised. These measures do not currently form part of recent translocation guidelines (e.g. the IUCN *Position Statement on Translocation of Living Organisms*, 1987; the IUCN *Guidelines for Re-introductions*, 1995; JNCC *Policy for Conservation Translocations of Species in Britain*, 2003). Monitoring for changes after translocation is advised, but attempting to minimise phenotypic and genetic changes through initial translocation planning and practices and has not yet formed an integral part of these documents. It is important that this becomes addressed.

In conclusion, while the establishment of refuge populations should not be used in preference to preservation of native populations or improvement of their habitat, in some cases they may be the only management option available. Where high conservation value or isolated populations of freshwater fish are threatened in their own environment, refuge populations become particularly important. In this case the creation of refuge populations provides a numerical barrier to extinction and is the best method for conserving life history and genetic diversity from catastrophic events.

Chapter 5 HAS HABITAT HETEROGENEITY PROMOTED PHENOTYPIC AND ECOLOGICAL SUB-STRUCTURING AMONGST A *COREGONUS LAVARETUS* POPULATION IN A LARGE SCOTTISH LAKE?

5.1 ABSTRACT

Sympatric morphotypes are found in many fish lineages, particularly those inhabiting postglacial lakes. These 'morphs' tend to specialise on a particular food resource or habitat and so sub-divide available resources. Loch Lomond represents an apparent candidate system to support such sub-structuring and at least one historical report suggested its existence. This large Scottish lake is divided into three basins, the north is reminiscent of a highland loch (deep, narrow, oligotrophic), while the south basin has the characteristics of a lowland loch (shallow, wide, mesotrophic); these are divided by an intermediate mid basin. Differences in stable isotope values, morphology and ecology in powan (*Coregonus lavaretus*) were investigated between the three basins. The results are discussed with reference to a genetic investigation to elucidate any sub-structuring or spawning site fidelity. Apart from some evidence of maintained feeding in certain areas of Loch Lomond indicated by $\delta^{13}C$ and $\delta^{15}N$ values of powan muscle tissue, there appears to be no evidence of the existence of sympatric morphs or sub-structuring in the whitefish population. A previous report of two powan 'species' in Loch Lomond are likely to reflect natural variation between individuals within a single mixed population.

5.2 INTRODUCTION

Sub-structuring in populations of fishes has been frequently recorded, particularly in postglacial lakes (Skulason & Smith, 1995; Smith & Skulason, 1996). At its most extreme, this takes the form of sympatric polymorphism, e.g., in some Arctic charr (*Salvelinus alpinus*) populations morphs can show discrete variation in morphological, meristic, behavioural, ecological, and life history traits (Jonsson & Jonsson, 2001). In lakes, habitats and feeding resources available to fish are relatively discrete and can be divided horizontally from the littoral to the pelagic and vertically by depth. Fish such as Arctic charr frequently show sub-structuring based on the habitat and food resources. Commonly this polymorphism takes the form of two groups of ecological specialists, such as a benthic and a pelagic feeder. Similar sub-structuring patterns are also known in other fish taxa such as pumpkinseed sunfish (*Lepomis gibbosus*) (Robinson & Wilson, 1996), percids (*Percichthys trucha*) (Ruzzante *et al.,* 1998) and three-spined stickleback (*Gasterosteus aculeatus*) (Baker *et al.,* 2005). More subtle structuring, such as genetic and morphological

sub-populations can also be found e.g. in Atlantic salmon (*Salmo salar*) (Verspoor & Cole, 1989).

Population sub-structuring has also been demonstrated in the European whitefish (*Coregonus lavaretus*) (Naesje *et al.*, 2004; Ostbye *et al.*, 2005b; Kahilainen & Ostbye, 2006). Some whitefish populations can support up to five sympatric morphs (Svardsen 1979; Bergstrand, 1982). Apart from habitat and feeding, they most often differ in body size (small and large body forms), growth and gill raker number (Bergstrand, 1982; Naesje *et al.*, 2004). Similar patterns of divergence are found in separate lineages of whitefish and in different lakes (see Chapter 1) (Douglas *et al.*, 1999, 2005).

Loch Lomond is the largest body of fresh water in Britain (ca. 71km^2) and was created by glacial encroachment from the north. It has many inflows, the largest of which are the River Falloch in the north and the Endrick Water in the south. Loch Lomond has a single outflow formed by the River Leven in the south. Loch Lomond is separated into a north basin and south basin divided by an intermediate mid basin. The north basin has the characteristics of a typical highland Scottish lake, i.e. it is narrow and deep (max. depth 190 m), is permanently thermally stratified in the summer months, it is surrounded by hard and often peat covered metamorphic rocks which yield little in the way of soluble nutrients and the waters of the north are therefore oligotrophic. The south basin has the characteristics of a typical lowland Scottish lake, i.e. it is wide and shallow (max. depth 30 m), has very weak and temporary thermal stratification in the summer months, it is surrounded for the most part with soft sedimentary rocks and fertile soils. The catchment is dominated by improved farmland and is relatively densely populated, therefore the waters of the southern basin are more mesotrophic. Moreover, the south basin with its myriad islands provides a heterogeneous environment (for more details see Appendix 1).

C. lavaretus is naturally extant in only seven lakes within the UK. Despite its rarity when compared to other species of freshwater fish, it is referred to locally as powan (Scotland), schelly (England) and gwyniad (Wales). Within Scotland *C. lavaretus* are found in only two locations, Loch Lomond and Loch Eck. The large size of Loch Lomond, with its multiple basins of very distinct nature and the high level of habitat heterogeneity would suggest that sub-structuring within populations is highly likely. Previous studies of British *C. lavaretus* populations suggested the presence of two types of gwyniad in Llyn Tegid, Wales (Dottrens, 1959). Studies of another coregonid species, the Irish pollan (*C. autumnalis*) revealed the existence of genetic sub-structuring in the pollan population of

Lough Neagh (Ferguson, 1975 and current work by Bradley *et al.*, Pers. Comm.). The presence of two distinct powan species in Loch Lomond was first postulated by Parnell (1838). These early observations suggested that two distinct species, differing primarily in head morphology: the long nosed powan (*C. lacepedei*) and the short head powan (*C. microcephalus*) existed within the loch. Therefore, there is good reason to expect sub-structuring in the Loch Lomond powan.

Population sub-structuring within a single species may have a number of important implications. Firstly, sympatric populations are important for the study of speciation. Secondly, differentiation within the population may complicate management and conservation of this protected species. For instance, different forms may face uneven susceptibility to pernicious impacts on a particular habitat, spawning site or food resource. Finally there is the impact on conservation action. Refuge populations created from the Loch Lomond powan were based on materials collected from a single spawning site. If multiple powan forms co-exist and they show assortive spawning, there is the possibility that not all diversity in the Loch Lomond powan is represented in these refuge populations. In order to examine the existence of multiple forms of powan in Loch Lomond, the null hypothesis that there are no significant differences (in ecology, life history and morphology) between powan caught in different areas of Loch Lomond is tested.

5.3 METHOD

5.3.1 Fish collection

Multi-panel benthic Nordic-pattern gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the three basins of Loch Lomond over the winter of 2005/06 (for additional site details see Appendix 1). Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986). In total, 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (9 November 2005 to 24 January 2006). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds. During this period a total of 118 powan were caught (north basin n = 25, mid basin n= 47, south basin n = 46). Fish were frozen (-20 °C) within four hours of capture (for additional details see Appendix 2).

5.3.2 Catch data

Data from nets set outside of the month when the most powan were caught (between 30/12/05 and 24/1/06) were excluded from CPUE analysis. Nets set at sites where no powan were ever caught were excluded. Fish catch rate was calculated as CPUE (catch-per-unit-effort), determined as the number of powan caught per 12 hours per net. The CPUE was compared between different basins using Kruskal-Wallis and Mann Whitney-U tests. All statistical analysis was performed using SPSS v.13.

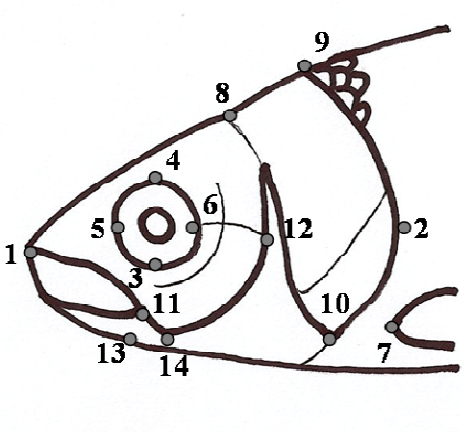
5.3.3 Stable isotope analysis

In the laboratory, fish were defrosted, a small piece of white muscle posterior to the head and above the lateral line was removed for stable isotope analysis. Tissue was dried at constant temperature (50°C for at least 48 hours), ground to a fine powder using a grinder (Revel Ltd.) and 0.5 mg of dried ground muscle was packed into pressed 10x10 mm tin cups and used in simultaneous analysis of stable C and N isotopes. Stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry at the Max Planck Institute for Limnology, Germany. Stable isotope ratios are given using the δ notation expressed in units per mil where δ (‰) = [(R sample/R standard)-1] x 1000, and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The reference materials used were secondary standards of known relation to the international standards of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Typical precision for a single analysis was ± 0.1 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. Comparisons of mean C:N values between basins indicated little need for adjustment of $\delta^{13}C$ values due to variation in lipid concentrations (Kiljunen *et al.*, 2006).

5.3.4 Head morphology

Digital photographs of the left side of the fish were taken using a Nikon Coolpix digital camera, attached to a copy stand with sufficient lighting and a suitable scale. Thin Plate Spline (TPS) is a landmark-based geometric technique for the determination of shape. The method provides a powerful analysis of shape independent of size (Rohlf, 1990, 2002; Bookstein, 1991) (morphometrics software downloaded from http://life.bio.sunysb.edu/morph). For the analysis of morphology, images of 14 individuals from each area were chosen that gave good detail of the head. A total of 14 landmarks (Fig. 5.1.) were identified on the head and digitised to coordinates using the programme TpsDig. Procrustes superimposition in the programme Coordgen6, with landmarks 1 and 2 as baseline end-points, was used to rotate, translate and scale procrustes coordinates. Based

on the obtained procrustes coordinates, new shape variables, known as partial warp scores were calculated in the programme PCAGen. These new variables capture spatial variation in a sample and can be used in statistical analysis. The partial warps were reduced by principal components analysis (PCA) and MANOVA was performed on the resulting component scores to examine whether shape differed between the areas of origin of the fish.



Landmark	Description
1	Snout
2	Most posterior point of operculum
3 - 6	Eye
7	Base of pectoral fin
8, 12, 14	Anterior side of operculum; dorsal, mid and ventral points
9, 10	Posterior side of operculum; dorsal and ventral points
11	Edge of maxillary
13	Depth of lower jaw under maxillary

Figure 5.1 14 landmarks used to examine variation in head shape of powan

5.3.5 Determination of size and age

Fork length (L_f) (to 1 mm) and weight (W) (to 0.01 g) were measured. Scales were removed from the flank below the dorsal fin for age determination. The clearest three scales were selected and impressed onto plastic. A projection microscope was then used to determine age, and the median age (determined from three scales) was taken as the age of the individual in order to minimise any impact of reading errors. The specimens were dissected and sex was determined by the visual examination of gonads. The first gill arch was dissected out and the gill rakers were counted. The outer surface of the stomach and gut was examined for cysts of the parasite *Diphyllobothrium*, and scored for the number of cysts of each individual fish as follows: 0 = 0, 1 = 1-9, 2 = 10-19, 3 = 20-50, 4 = 50+. The adipose fin was removed and stored in 100% ethanol. Genetic work on this tissue took place in the Fisheries Research Services laboratory in Pitlochry and has been reported on by Thompson *et al.* (2008).

5.4 RESULTS

5.4.1 Catch rate

The catch rate of powan was significantly different between basins of Loch Lomond (Kruskal Wallis, d.f. = 2, $p < 0.01$). The CPUE was significantly lower in the north compared with the mid basin (Mann-Whitney U, $p < 0.01$), but there was no significant difference in the CPUE between the north and south (Mann-Whitney U, $p = 0.09$), and the mid and south basins (Mann-Whitney U, $p = 0.12$) (Fig. 5.2).

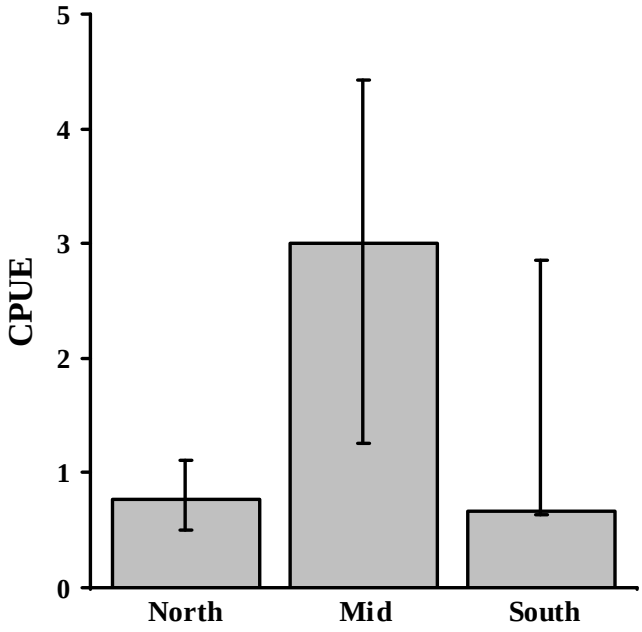


Figure 5.2 Median Catch per Unit Effort of powan from the north, mid and south basins of Loch Lomond. Lower bars indicate 25 % percentile, upper bars indicate 75 % percentile

5.4.2 Stable isotope analysis

There was a significant difference in mean powan muscle tissue $\delta^{13}C$ values between basins (ANOVA, $F_{2,112} = 5.1$, $p < 0.01$) Bonferroni *post hoc* comparisons showed that the powan from the mid basin were significantly more depleted in $\delta^{13}C$ than powan from the south basin ($p < 0.05$), but there was no significant difference between powan from both the north and mid basins ($p = 0.06$) and the north and south basins ($p > 0.99$). There was also a significant difference between basins in muscle $\delta^{15}N$ values (ANOVA, $F_{2,112} = 17.7$, $p < 0.0001$). Bonferroni *post hoc* comparisons showed that the powan from the south basin were significantly more enriched in $\delta^{15}N$ than powan from both the north ($p < 0.0001$) and the mid basins ($p < 0.0001$), but there was no significant difference between powan from

the north and mid basins (p = 0.49). These differences remained significant when two outliers were removed (Fig. 5.3.). Across all sites there was however an almost significant difference between sexes in $\delta^{15}N$ of muscle tissue (t-test, t = 2.2, d.f. = 12, p = 0.05) with males having being more $\delta^{15}N$ enriched than females, but no significant difference between sexes was found in $\delta^{13}C$ (t-test, t = 0.79, d.f. = 12, p = 0.45).

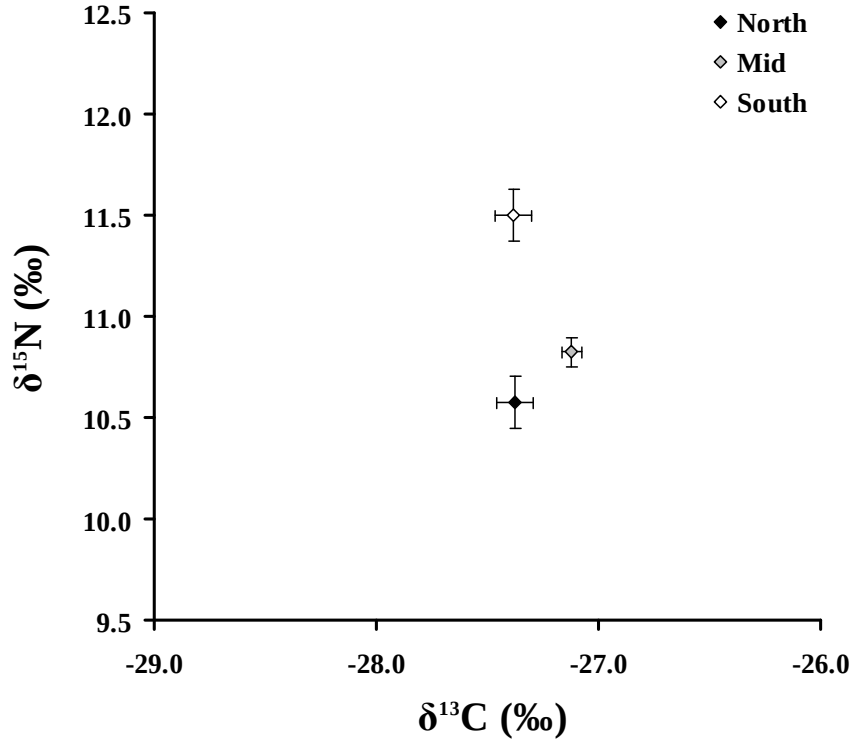


Figure 5.3 Variation in mean (± S.E.) $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue from powan from the north, mid and south basins of Loch Lomond

Across all sites there was a significant negative correlation between age and $\delta^{15}N$ (Pearson correlation = - 0.35, $p < 0.001$) and a significant positive correlation between age and $\delta^{13}C$ (Pearson correlation = 0.44, $p < 0.0001$). However, further examination found that individually among sites there was only a significant negative correlation between age and $\delta^{15}N$ (Pearson correlation = - 0.38, $p < 0.05$) in the south basin, and a significant positive correlation between age and $\delta^{13}C$ in the mid (Pearson correlation = 0.49, $p < 0.001$) and south basins (Pearson correlation = 0.43, $p < 0.01$). When powan less than 4+ years were removed from the analysis, only the significant positive correlations between age and $\delta^{13}C$ in the mid and south basins remained.

5.4.3 Head morphology

PC1 of the partial warp scores explained 17.0 % of the total variation in landmark position. Fish with an extreme positive PC1 score were typified by a more posteriorly placed dorsal anterior and posterior edge of the operculum, the pectoral fin slightly more anterior and the eye slightly more dorsal, compared with an extreme negative PC1 score. Differences in PC1 scores between the three areas of Loch Lomond were not significant (ANOVA, $F_{2,39}$ = 0.35, p = 0.97). PC2 of the partial warp scores explained 13.8 % of the variance seen in landmark position. Fish with an extreme positive PC2 score were typified by a deeper head, more posterior pectoral fin and a more anterior eye, compared with an extreme negative PC2 score. Differences in PC2 scores between powan from the three basins were not significant (ANOVA, $F_{2,39}$ = 0.63, p = 0.54) (Fig. 5.4.).

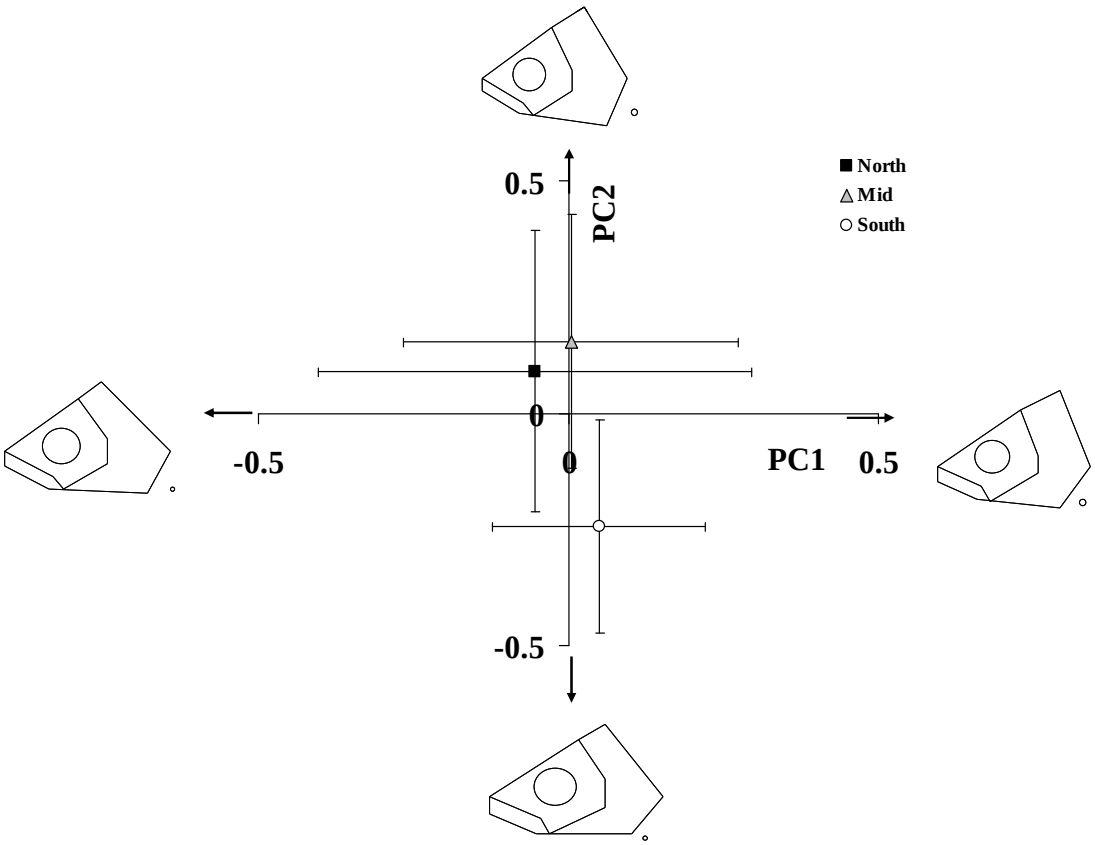


Figure 5.4 Variation in mean (± S.E.) PC1 and PC2 scores describing the head of powan from north, mid and south basins of Loch Lomond

There was also no significant difference in number of gill rakers (ANOVA, $F_{2,73}$ = 1.8, p = 0.18) between powan from the three basins in Loch Lomond (Fig. 5.5.). However, it was interesting to note that there was a significant difference between sexes in PC1 scores (t-test, t = 3.3, d.f. = 37, p < 0.01) with males having a significantly greater PC1 score than

females. No significant difference between sexes was found in PC2 scores (t-test, t = 0.20, d.f. = 37, p = 0.85).

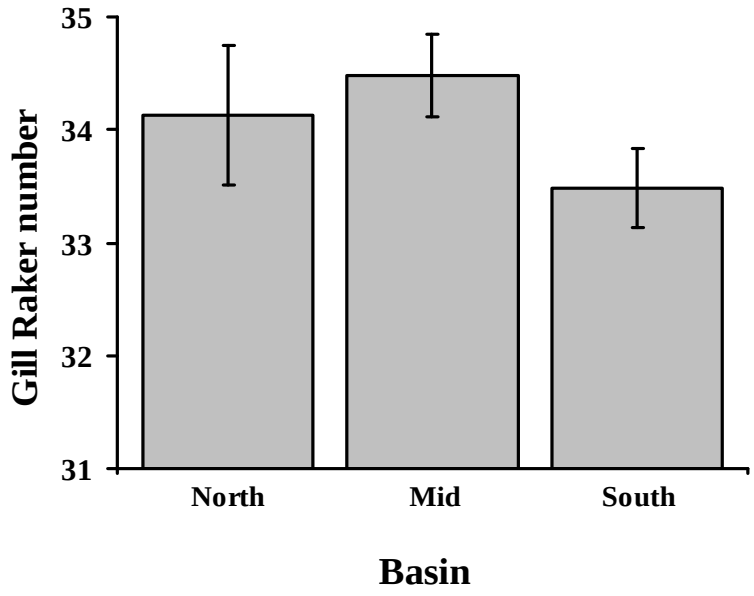


Figure 5.5 Mean (± S.E.) gill raker numbers of powan from north, mid and south basins of Loch Lomond

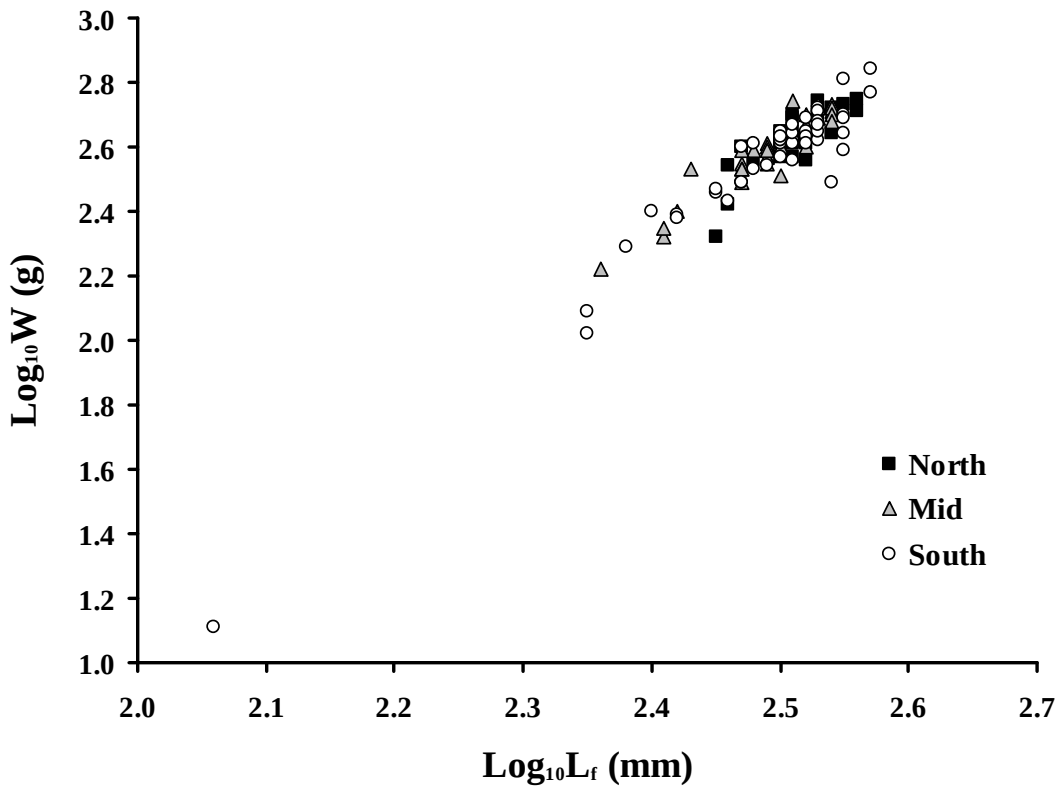


Figure 5.6 Relationship between $Log_{10}L_f$ and $log_{10}W$ of powan from north (y = 2.7x - 4.2), mid (y = 2.7x – 4.1) and south (y = 3.2x - 5.3) basins of Loch Lomond

5.4.4 Life history

There was no significant difference in L_f (Kruskal-Wallis = 1.8, d.f. = 2, p = 0.40) or weight (Kruskal-Wallis = 1.6, d.f. = 2, p = 0.45) between basins. The relationship between $\log_{10}L_f$ and $\log_{10}W$ could be described with a linear relationship in north (ANOVA, R^2 = 0.67, $F_{1,23}$ = 46.2, p < 0.0001), mid (ANOVA, R^2 = 0.89, $F_{1,46}$ = 371.7, p < 0.0001) and south basins (ANOVA, R^2 = 0.94, $F_{1,44}$ = 661.6, p < 0.0001) (Fig. 5.6.). There was a significant difference between linear relationships between sites (ANCOVA, R^2 = 0.92, $F_{2,114}$ = 3.3, p < 0.05), however, when an outlier was removed (the smallest fish) there was no longer a significant difference (ANCOVA, R^2 = 0.84, $F_{2,113}$ = 2.9, p = 0.06). Powan samples from the south basin of Loch Lomond had a lower mean age resulting in a near significant difference in age between basins (Kruskal-Wallis = 6.0, d.f. = 2, p = 0.05). This was the only part of Loch Lomond where powan less than 4+ yrs of age were caught. If powan below this age were removed from analysis there was no significant difference between basins (Kruskal-Wallis = 3.1, d.f. = 2, p = 0.21). The most frequent age of powan in all areas of Loch Lomond was 6+ years. L_f at age of powan from the north ($F_{1,22}$ = 9.8, R^2 = 0.47, p < 0.001), mid ($F_{2,44}$ = 81.6, R^2 = 0.79, p < 0.0001) and south ($F_{2,42}$ = 124.6, R^2 = 0.86, p < 0.0001) basins of Loch Lomond were best described with a quadratic relationship (Fig. 5.7.). The resulting curves were not significantly different (F-test, $F_{2,114}$ = 2.4, p = 0.10).

There was no significant difference in scores for infection intensity of *Diphyllobothrium spp*. in powan between basins of Loch Lomond (Kruskal Wallis, d.f. = 2, p = 0.18) (Fig. 5.8.). Additionally there was no significant difference between the prevalence of infected powan between the north (prevalence = 100 %), mid (100 %) and south (98 %) of Loch Lomond (χ^2 = 1.6, d.f. = 2, p = 0.45).

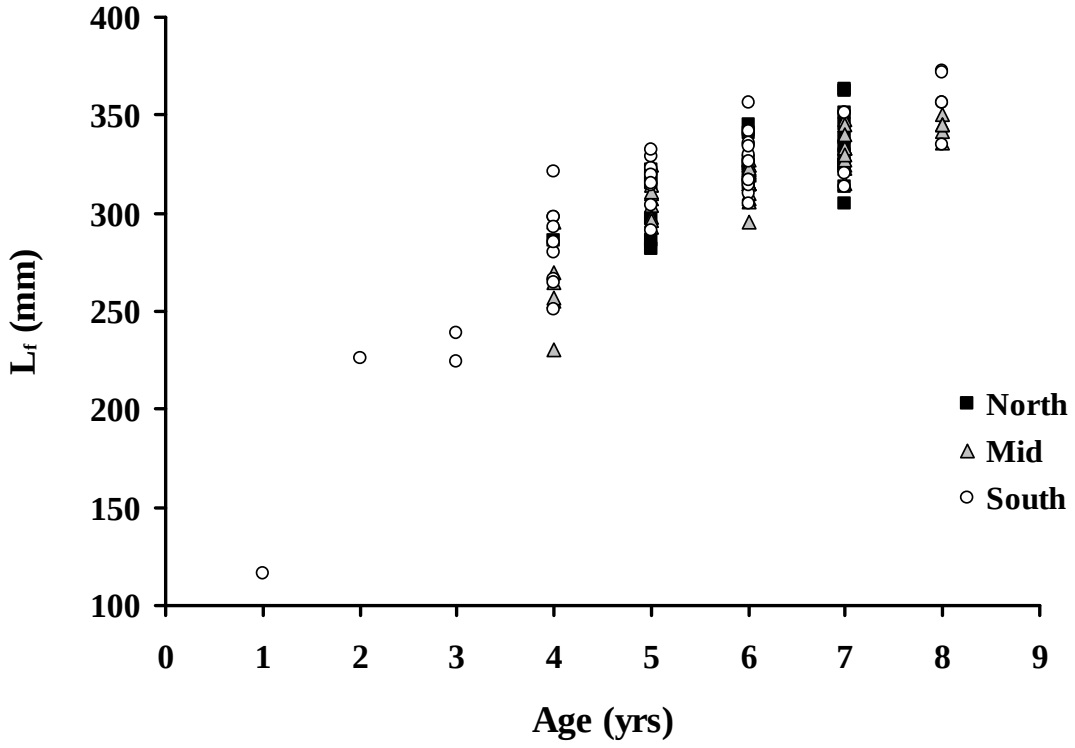


Figure 5.7 Relationship between L_f at age of powan from north ($y = 67.8x - 4.5x^2 + 79.6$), mid ($y = 64.4x - 3.9x^2 + 71.5$) and south ($y = 68.0x - 4.2x^2 + 76.3$) basins of Loch Lomond are best described with a quadratic model

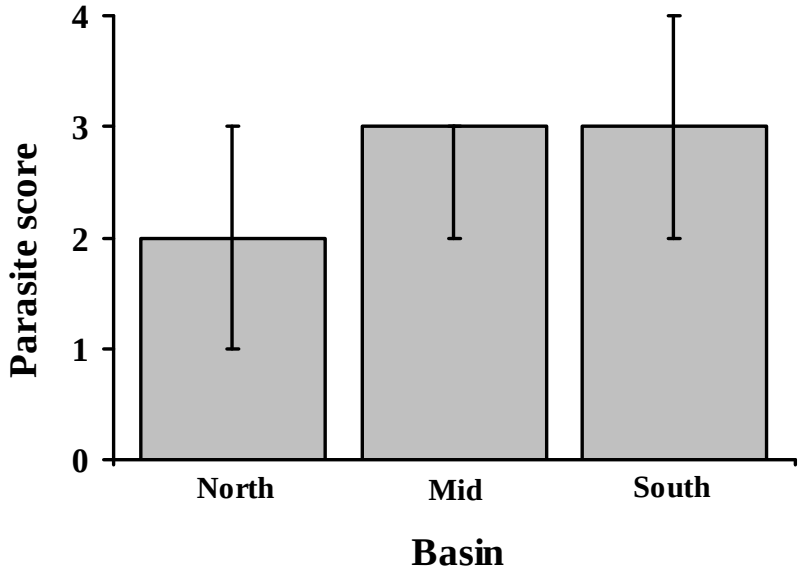


Figure 5.8 Median parasite scores of powan from north, mid and south basins of Loch Lomond. Lower bars indicate 25% percentile, upper bars indicate 75% percentile

5.5 DISCUSSION

Morphological, ecological and genetic sub-structuring of fishes in postglacial northern hemisphere lakes is relatively common. In the UK a significant number of Arctic charr (*S. alpinus*) populations show evidence of sub-structuring in the gene pool, morphology, ecology and life history (Adams *et al.*, 1998; McCarthy *et al.*, 2004; Wilson *et al.*, 2004; Garduño-Paz, 2009). Where sub-structuring takes the form of sympatric morphs, these often show divergence into pelagic forms foraging on plankton with associated morphological and meristic specialisation and benthic forms specialising in foraging on macrobenthos (Schluter & McPhail, 1993; Robinson & Wilson, 1994). *C. lavaretus* is highly variable across its range. This species can show genetic structuring between and within lakes, with multiple sympatric morphs reported from many lakes ranging throughout the distribution of the species (Svärdson, 1957; Bergstrand, 1982). The polymorphism in this species often takes the form of differentiation in phenotype, ecology, life history (Bergstrand, 1982; Amundsen, 1988; Kahilainen & Ostbye, 2006) and morphs can represent discrete gene pools (Naesje *et al.*, 2004; Ostbye *et al.*, 2005b). Amongst the pollan (*C. autumnalis*) in Lough Neagh, Northern Ireland, genetic sub-structuring has been shown (Ferguson, 1975 and current work by Bradley *et al.*, Pers. Comm.), though this has not been associated with any ecological polymorphism.

The isotopic turnover of muscle depends on growth rate, but typically is considered to be ca. 6 months (Perga & Gerdeaux, 2005; Phillips & Eldridge, 2006). Significant differences in $\delta^{13}C$ and $\delta^{15}N$ of fish sampled from different areas of Loch Lomond, indicated differences in trophic ecology of powan from different parts of the loch. $\delta^{13}C$ values closely reflect the origin of the carbon source the organism has been assimilating, due to limited fractionation, trophic enrichment in $\delta^{13}C$ is typically ca. <1‰ (Peterson & Fry, 1987; Hobson, 1999). Enriched $\delta^{13}C$ values are indicative of atmospheric C, while depleted $\delta^{13}C$ values are indicative of endogenous (recycled) C sources. Small but significant differences in $\delta^{13}C$ of powan muscle tissue suggest that powan in the mid basin may be specialising on $\delta^{13}C$ enriched prey such as littoral macroinvertebrates (France, 1995; Post, 2002). Alternatively, the different natures of the loch basins may lead to different isotopic baselines which in turn are reflected in powan muscle tissues, for instance $\delta^{13}C$ values in a water body can be influenced by riverine inputs, the surface area of a water-body and eutrophication (Post, 2002; Perga & Gerdeaux, 2004). However, in Loch Lomond levels of dissolved organic C are generally greater in the south basin (Bass, 2007). Trophic enrichment in $\delta^{15}N$ is typically ca. 3-5‰, this allows the long-term trophic position of consumers to be estimated (Peterson & Fry, 1987; Post, 2002; Sweeting *et al.*, 2007).

Significant differences in $\delta^{15}N$ of powan muscle tissue initially suggest that powan from the south basin are feeding at a higher trophic level. However, the small differences recorded in powan $\delta^{15}N$ may also reflect isotopic differences at the base of the food webs in the different basins. For instance $\delta^{15}N$ values in a water body can be increased by anthropogenic input of nutrients (Constanzo *et al.*, 2001). Certainly $\delta^{15}N$ values of total dissolved N in Loch Lomond are greatest in the south, which was where powan were most enriched in $\delta^{15}N$ (Bass, 2007). Therefore it is believed that the distinction in $\delta^{15}N$ is due to between-basin baseline differences rather than trophic ecology.

While the stable isotope values of powan muscle tissue can be interpreted as fish feeding on different prey items, powan in Loch Lomond have been previously described as specialising on plankton (Pomeroy, 1991) which is supported by the high *Diphyllobothrium* load of these fish. Therefore, it seems more likely that these isotopic differences reflect the discrete natures of the north and south basins as opposed to differences in the diet of powan in these areas. This in turn suggests that there is foraging fidelity between basins. While the overlap between individuals suggests that this is not complete, individuals apparently feed in a given basin for months in order to develop the spatial differences in stable isotope values in their muscle tissues.

Head morphology is associated with efficiency of capturing and processing food and therefore may reasonably be expected to be different when individual fish specialise on particular diets (Gardner *et al.*, 1988; Adams & Huntingford, 2002). There were no significant differences in head shape between fish caught in the different areas of Loch Lomond. There was however, a significant difference in head morphology between males and females. Since only the head was examined for shape differences, body shape changes in females during spawning could not be the cause of this difference. There was also an almost significant difference in $\delta^{15}N$ enrichment between males and females. However, it is difficult to draw firm conclusions from this due to the small sample size of females (7 females, 32 males, 3 unknown for morphology; 11 females, 99 males, 7 unknown for stable isotope analysis) which due to the behaviour of powan are usually under represented in catches on spawning grounds (Brown & Scott, 1994). Gill raker number is also linked with different diets; a benthic diet is typically associated with low number of gill rakers, while a pelagic diet is typically associated with a high number of gill rakers (Amundsen, 1988; Lehtonen & Kahilainen, 2002). However, there was no significant difference in gill raker number between fish caught in the different areas of Loch Lomond. Particular parasite infections are also associated with certain diets and elevated infection intensities of

Diphyllobothrium infection are associated with zooplankitvory (Amundsen & Klemetsen, 1988; Dolezel & Crompton, 2000). There was no significant difference in parasite load between fish caught in the different areas of Loch Lomond suggesting a similar dependence on pelagic resources. There was also no significant difference in size or growth between fish caught in the different areas of Loch Lomond, providing further evidence for a lack of spatial segregation in the powan population (c.f. with Kahilainen *et al.*, 2003). Taken together, these data strongly suggest that there is no phenotypic sub-structuring within powan in Loch Lomond, but that there is spatial foraging segregation for a period of months where powan from each basin forage to a large extent within that basin.

Utilising six microsatellites, Thompson *et al.*, (2008) used mean allelic richness as a measure of genetic diversity: they found mean allelic richness values of 4.9 in the north basin, 4.8 in the mid basin and 4.7 in the south basin of Loch Lomond. Using these data I found no significant difference in genetic diversity between basins (ANOVA, $F_{2,15} = 0.005$, $p > 0.99$). Thompson *et al*. (2008) also showed that there are no detectable genetic differences between powan from the north and mid ($p = 0.76$), north and south ($p = 0.42$) and mid and south ($p = 0.56$) basins of Loch Lomond. Thus, the apparent fidelity in foraging site is not reflected in any genetic segregation and the genetic and morphological information suggests a panmictic population with no spawning site fidelity. An alternative explanation is that foraging site fidelity does not result in genetic sub-structuring of the population because of a high straying rate, where a number of basin-specific individuals foraging in one basin contribute to spawning in another basin.

The significantly lower catch rate in the north basin of Loch Lomond is probably due to the limited availability of suitable spawning grounds in this basin, which is very steep sided and has few shallow areas (Slack, 1955). However, there was a difference in age structure between basins, with young fish only being found in the south. Since I sampled during the spawning period, I did not expect to capture immature fish. While it is possible that the south basin is a better habitat for young fish, it may simply be that immature fish are less likely to be caught on the spawning grounds in the mid and north basins. However, since this result was influenced by the capture of a very small number of young fish, perhaps by chance, firm conclusions cannot be drawn. In the $\delta^{15}N$ values there were several outliers including two fish that appeared to be feeding up to one trophic level above and below the others. The one fish apparently feeding at the highest trophic level was the youngest (1 year), there is a negative correlation between age and $\delta^{15}N$ suggesting that there is an

ontogenic shift in feeding. This is common in many fish species: juveniles are completely zooplanktivorous, while adults include some benthos in the diet. It has been suggested that these changes are more likely to occur in a monomorphic population to avoid infraspecific competition between age classes (Hessen *et al*., 1986; Sandlund *et al*., 1992).

In conclusion, I show no evidence of sub-structuring or trophic polymorphism in Loch Lomond powan, despite the size and complexity of this lake, often considered a prerequisite for the formation of sub-structuring. Although Parnell (1838) described two distinct powan species in Loch Lomond, his description was based on only two specimens. According to Day (1884), Parnell later received intermediate forms of these fish suggesting that these fish reflected natural variation between individuals within a single mixed population. Although Dottrens (1959) similarly proposed the existence of multiple forms of *C. lavaretus* in the single natural Welsh population in Llyn Tegid their presence has never been confirmed, even following considerable examination, and is likely a mistake (Gasowska, 1965; Haram, 1968). The lack of sub-structuring in Loch Lomond may reflect the species-rich nature of the lake (Adams, 1994). While powan are likely to have been amongst the first invaders into Loch Lomond following the last glaciation and may therefore have initially undergone population structuring, the subsequent invasion of other fish species filling the available niches probably precluded this. Importantly from a conservation perspective, powan collected from one area of Loch Lomond are likely to be representative of the whole population.

Chapter 6 SUBSTRATE SPECIFIC VULNERABILITY OF POWAN (*COREGONUS LAVARETUS*) OVA TO PREDATION BY RUFFE (*GYMNOCEPHALUS CERNUUS*).

6.1 ABSTRACT

European whitefish (*Coregonus lavaretus*) eggs are vulnerable to a variety of mortalities, with substrate on spawning grounds providing the only physical protection. The Loch Lomond population of *C. lavaretus* is believed to be adversely affected by invasive ruffe (*Gymnocephalus cernuus*) predation on eggs. In order to discover the protective ability of substrate commonly found on whitefish spawning grounds, predation experiments of ruffe on artificial eggs were conducted. These were presented to ruffe over different substrates: sand, gravel, pebbles and cobbles. It was found that the greatest protection is provided by pebbles and gravel. Eggs are exposed on sand, but are protected by small gaps between pebbles and gravel, while in cobbles the gaps between substrate particles are large enough to sometimes allow ruffe to foraging within the substrate. Using these results, a comparison between the potential protective ability of substrates of spawning grounds in four Scottish whitefish sites was attempted.

6.2 INTRODUCTION

The whitefish *Coregonus lavaretus* despite being locally common in some areas of northwest and central Europe is one of the rarest species of freshwater fish in Britain (Maitland & Lyle, 1991). *C. lavaretus* is considered to be particularly vulnerable within Britain and it is protected under Schedule 5 of the Wildlife and Countryside Act 1981 and is listed as a priority species within the UK Biodiversity Action Plan (UKBAP). There are only seven extant native populations found in Britain, of which two are located in Scotland, in Loch Lomond and Loch Eck. As a conservation measure, two refuge populations of Loch Lomond origin whitefish were founded in Loch Sloy and Carron Reservoir (Maitland & Lyle, 1992). There is evidence of a recent decline in the abundance of whitefish in Loch Lomond (see Chapter 3) (Winfield *et al*., 2005, 2008). There is no evidence of any similar decrease in the only other native population of Scottish whitefish in Loch Eck, a site located 20 km to the west of Loch Lomond (Winfield *et al.*, 2006, 2008; Etheridge, 2009). This suggests that the observed decline is not due to wide-ranging climatic factors but more likely due to local, within-lake factors.

The key difference between the two sites is the introduction of ruffe (*Gymnocephalus cernuus*), a pernicious non-native benthic feeding fish, first recorded in Loch Lomond in

1982 (Maitland *et al.*, 1983). This species has been accidentally introduced into water bodies, in the USA (Pratt *et al.*, 1992), mainland Europe (Rosch & Schmid, 1996) and Britain (Maitland *et al.*, 1983; Winfield *et al.*, 1996). Invasive species such as ruffe can have important and sometimes unforeseen impacts on trophic interactions in the invaded community, i.e. since establishment they have formed an important part of the feeding regimes of many predatory species in Loch Lomond (see Appendix 3) (Adams, 1991; Adams & Mitchell, 1995; McCafferty, 2005). In addition, ruffe have been found to feed extensively on whitefish eggs (Adams & Tippett, 1991).

Whitefish eggs have a high rate of mortality. While in many fish species, including other salmonids, eggs may be protected by guarding behaviour and the construction of nests (Sargent *et al.*, 1987), whitefish eggs are only protected by the substrate upon which they fall after broadcast spawning. Sources of mortality of whitefish eggs include being swept off spawning grounds into unsuitable habitat, burial or smothering (particularly by deposited sediments in eutrophic conditions) (Ventlingschwank & Livingstone, 1994), water level change (Winfield *et al.*, 2004), high temperatures (Slack *et al*., 1957; Trippel *et al.*, 1991), and predation by both invertebrates (Slack, 1955; Fox, 1978) and fish (Adams & Tippett, 1991; Pomeroy, 1991).

Whitefish spawn in the winter when many fish have depressed feeding rates which offers some protection from fish-mediated egg predation (Adams & Tippett, 1991). However, ruffe are relatively unaffected by low temperatures and their feeding rate remains high in winter (Bergman, 1987). Within their native range, ruffe are known to co-exist with *Coregonus spp*. and there are many instances where no apparent predation on fish eggs can be demonstrated (Ogle, 1995; Winfield *et al.*, 1996). Or where fish declines after the invasion of ruffe can be explained by other factors (Bronte *et al.*, 1998). There are however, many laboratory (Sterligova & Pavlovskiy, 1984 DeSorcie & Edsall, 1995) and field (Pokrovski, 1961; Balagurova, 1963; Titova, 1973; Mikkola *et al.*, 1979; Adams & Tippett, 1991; Huusko & Sutela, 1992; Rosch & Schmid, 1996; Selgeby, 1998) studies which suggest that egg predation by ruffe may have a significant negative impact on coregonid populations.

The effects of different substrate types and foraging habitat complexity can affect ruffe foraging efficiency (Mattila, 1992; Fullerton *et al.*, 1998; Dieterich *et al.*, 2004). However, it is not known what level of protection against ruffe predation is afforded to incubating whitefish ova by differing substrate types. In this study, the predation success of ruffe

foraging on whitefish eggs amongst different substrates is examined in laboratory experiments. Experimental data are then used to estimate egg vulnerabilities at known whitefish spawning sites in four different Scottish lakes.

6.3 METHODS

6.3.1 Ruffe capture

Four fyke nets were set in Loch Lomond, two between 9th June 2008 and 12th June 2008 and two between16th June 2008 and 20th June 2008. A total of 54 ruffe were captured. Upon transfer to the laboratory holding facilities, ruffe were fed on a mixture of frozen bloodworm and artificial whitefish eggs (see below), the proportion of artificial eggs was increased on a daily basis. They were kept in captivity for a minimum of five days before experimentation, by which time they were accustomed to feeding wholly on artificial eggs.

6.3.2 Artificial egg manufacture

Due to the high conservation value of whitefish and evidence of a decline in whitefish numbers, it was decided to use artificial eggs for the purposes of experimentation as opposed to taking these from spawning whitefish. Artificial eggs were made with homogenised bloodworm in a gelatine matrix of identical size to real whitefish eggs. Artificial eggs mimicked size, consistency and texture of real eggs and were stable for several hours in water.

6.3.3 Substrate

Sand (defined as 0.0625 - 2.0 mm), gravel (2.0 – 16.0 mm), pebble (16.0 – 64.0 mm) and cobble (64.0 – 256.0 mm) (Cummins, 1962; Coyle & Adams, 2008) were collected by hand from littoral areas of Loch Lomond. Enough of a substrate was utilised to cover the feeding tray (a 335 x 235 mm plastic tray) in one layer. Due to the size of the feeding tray and experimental tanks, cobbles were all $\leq$ 230 mm in length.

6.3.4 Visible gaps

The space between substrate particles open to the surface and thus available for eggs to fall into, will from this point forward be referred to as the ‘visible gap’. A single substrate type was used to cover a black-coloured floor of a feeding tray (described above). The tray was then photographed. The programme imageJ was used to quantify the total area of black on the image, equating to the area of ‘visible gap’, as a proportion of total area. This was repeated for each substrate type in turn and each substrate replicated 20 times in different configurations to calculate an average visible gap for each substrate type. Differences in

visible space were compared with Mann Whitney-U tests. Since the standard substrate size categories (sand, gravel, pebble cobble) increase exponentially, in analyses the mid-size of the category was transformed using $\log_e$.

6.3.5 Experimental design

Four tanks were arranged identically. One half of the tank was filled with plastic plants in identical positions, for cover; ruffe had been found feed more readily with available shelter. The other half was an open area in which the feeding tray could be placed in the same position in each tank. To avoid visual contact between groups, the back and side walls of the aquaria were masked with black plastic film. To minimise disturbance to foraging behaviour, a hide was built that enabled observation of the ruffe through during trials. Dieterich *et al.* (2004) found that lone ruffe did not feed in experiments, thus three fish were used in each trial. The three ruffe were introduced into each tank to acclimate for two days during which they were fed artificial eggs once a day on a feeding tray without substrate, the artificial eggs were introduced using the experimental method (see below). On the third day after introduction to the experimental tank, the trial for each group began.

During the trial, once per day each group was exposed to a randomly assigned substrate type. This was introduced on the feeding tray, upon which 30 artificial eggs (weighed before use) were allowed to fall without any falling outside the tray. This was enabled with the use of a rectangular funnel which fit tightly inside the tray. The artificial eggs were allowed to settle and the ruffe were then allowed access to the feeding tray. The ruffe were observed for 30 minutes during which general observations of behaviour were noted. After this time the sediment tray was removed and the 'egg survival', defined as the number of eggs remaining, was recorded. Fish were not fed outside the experiment leading to the expectation that all artificial eggs that could be consumed were consumed. The next day each experimental ruffe group was exposed similarly to one of the remaining three of four substrate types. This was repeated until each experimental ruffe group was exposed to each substrate type once over a four day period. The experimental groups of ruffe were not reused. A total of 18 trial replications were completed.

If no fish was observed to move onto the tray during all of the four days of the trial, the replicate was removed from analysis (two replicates were removed). The differences in egg survival between treatments were tested using a repeated measure ANOVA and Bonferroni *post hoc* testing. Learning was excluded as a factor by testing for correlation between egg

survival and day of trial. Differences in artificial egg size was excluded as a factor by testing for correlation between egg survival and weight of the 30 artificial eggs used in the experiment.

6.3.6 Substrate surveys

Transects perpendicular from the shore on known spawning sites of whitefish in four lakes in Scotland were competed using a Videoray Remote Operated Vehicle (ROV). At known intervals of the transect, percentage cover on the lake bed of different substrate types and organic material (i.e. macrophytes, organic debris) in the viewing area of the ROV was recorded, along with depth.

In total 16 transects were completed in the Ross Bay area of Loch Lomond (NGR 368 955). Ten transects were completed from the beach of Ross Bay (21st December 2007), these were 10 m apart on the shore line and point surveys were completed every 3 m. Four transects were completed from the east of Ross bay (10th and 11th January 2008), and two transects were completed from the west of Ross bay (24th January 2008). These transects were 20 m apart on the shore line and point surveys were completed every 5 m. Four transects were completed in Loch Eck (NGR 140 946) (10th February 2008). Transects were 20 m apart on the shore line and point surveys were completed every 3 m. Two transects were completed in Loch Sloy (NGR 285 112) (10th February 2008). Transects were 20 m apart on the shore line and point surveys were completed every 3 m. Three transects were completed in Carron Valley Reservoir (NGR 715 838) (12th February 2008). Transects were 20 m apart on the shore line and point surveys were completed every 3 m.

6.3.7 Estimation of predation risk

Data from the laboratory experiments on substrate specific predation risk was used as an index of predation risk. This was combined with actual spawning site substrate data for four known spawning sites to estimate relative predation risk in the natural environment. For each percentage cover location, predation risk for a particular substrate was multiplied by the proportional area covered by that substrate (organic material was excluded). This was summed to provide a total estimated predation risk at that location.

6.4 RESULTS

6.4.1 Foraging experiments

There was no correlation of egg survival (number remaining out of 30) of artificial eggs with day of trial (Pearson correlation, $p = 0.72$) or artificial egg weight (Pearson

correlation, p = 0.53). This suggested that there was no learning effect on foraging efficiency, or any preferential size of egg effect of predation. There was a significant difference between the survival of artificial eggs on the different substrates (repeated measures ANOVA, $F_{3,45} = 20.3$, $p < 0.0001$). Bonferroni *post hoc* testing found significant differences in survival of artificial eggs between sand and gravel, and sand and pebbles ($p < 0.0001$) and between sand and cobbles ($p < 0.05$), and between pebbles and cobbles ($p < 0.05$). However, there was no significant difference in egg survival between gravel and pebbles ($p > 0.99$), or gravel and cobbles ($p = 0.48$). The substrate from which the most artificial eggs survived (number remaining out of 30) was pebbles (mean 22.7 ± se 1.3, median 24.5), then gravel (mean 20.3 ± se 1.6, median 20.5), cobbles (mean 16.0 ± se 1.8, median 13.0) and the lowest survival (highest predation rate) from sand (mean 7.1 ± se 2.6, median 0) (Fig. 6.1.). The $\log_e$ substrate mid-size and the artificial egg survival were significantly correlated and can be best explained by a quadratic relationship ($R^2 = 0.39$, ANOVA, $F_{2,61} = 319.6$, $p < 0.0001$).

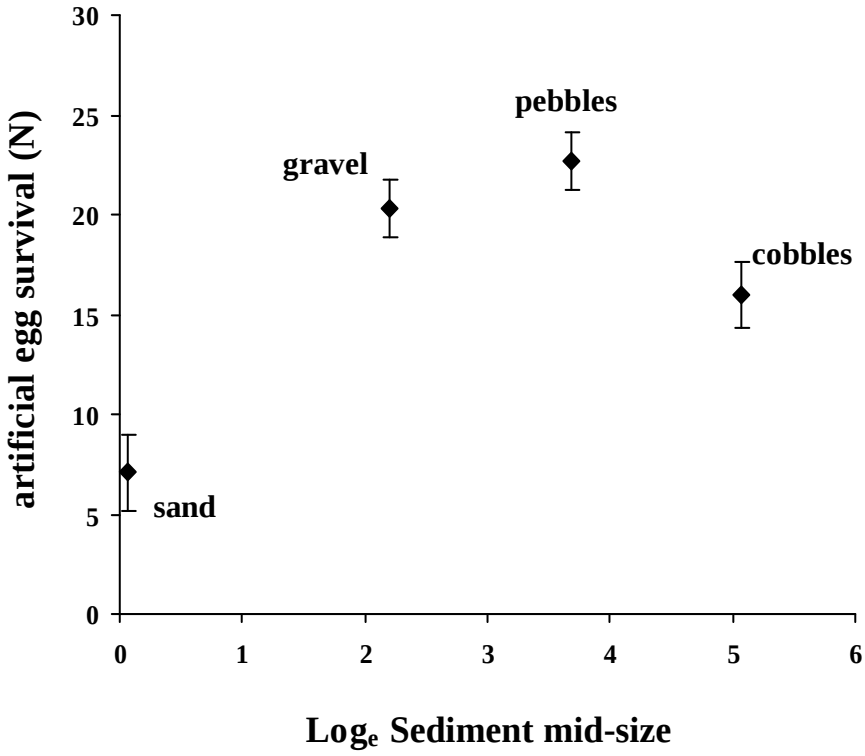


Figure 6.1 Mean (± S.E.) number of eggs removed from tray containing different sediments after ruffe foraging

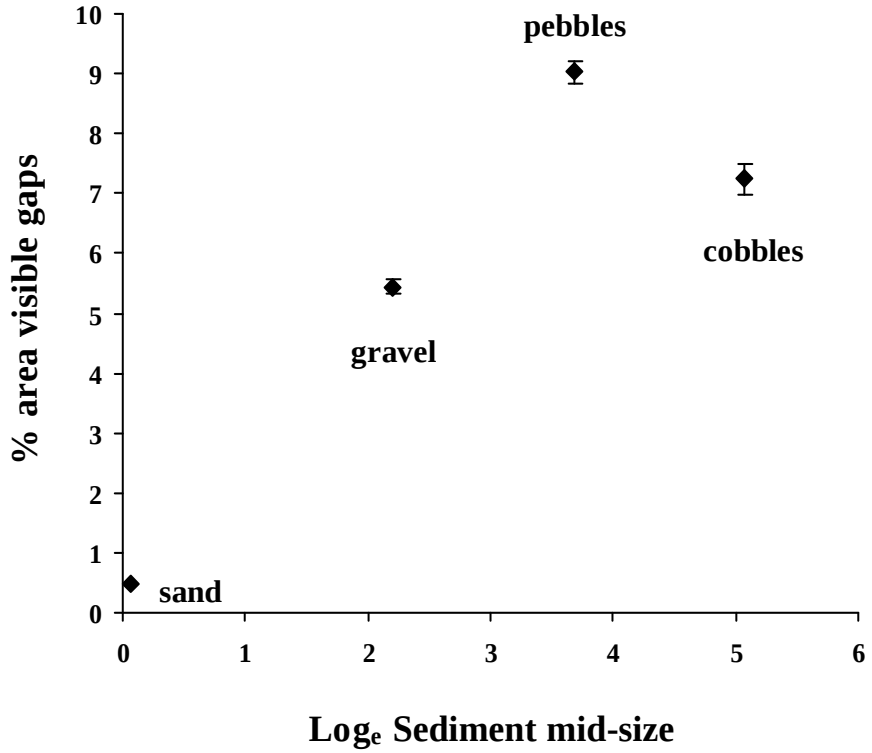


Figure 6.2 Mean (± S.E.) percentage area of visible gaps between sediment particles of sand, gravel, pebbles and cobbles

There was a significant difference in the percentage surface area comprising visible gaps (Kruskal-Wallis, χ^2 = 69.5, d.f. = 3, $p < 0.0001$). Mann-Whitney tests found significant differences between all pairwise comparisons ($p < 0.0001$). Mean percentage visible gaps were found to be, from the least to the greatest, sand (0.5 ± se 0.1 %), gravel (5.4 ± se 0.1 %), cobbles (7.2 ± se 0.3 %), pebbles (9.0 ± 0.2 %). The relationship between $\log_e$ mid-size of substrate and percentage of surface area comprising visible gaps can be best explained by a quadratic relationship (R^2 = 0.95, ANOVA, $F_{2,61}$ = 309.5, $p < 0.0001$) (Fig. 6.2.). There was a greater survival of eggs from substrates that have a greater percentage of surface area comprising visible gaps. The mean percentage of surface area comprising visible gaps and the surviving number of artificial eggs can be best explained by a cubic relationship (R^2 = 0.40, ANOVA, $F_{3,60}$ = 13.0, $p < 0.0001$) (Fig. 6.3.).

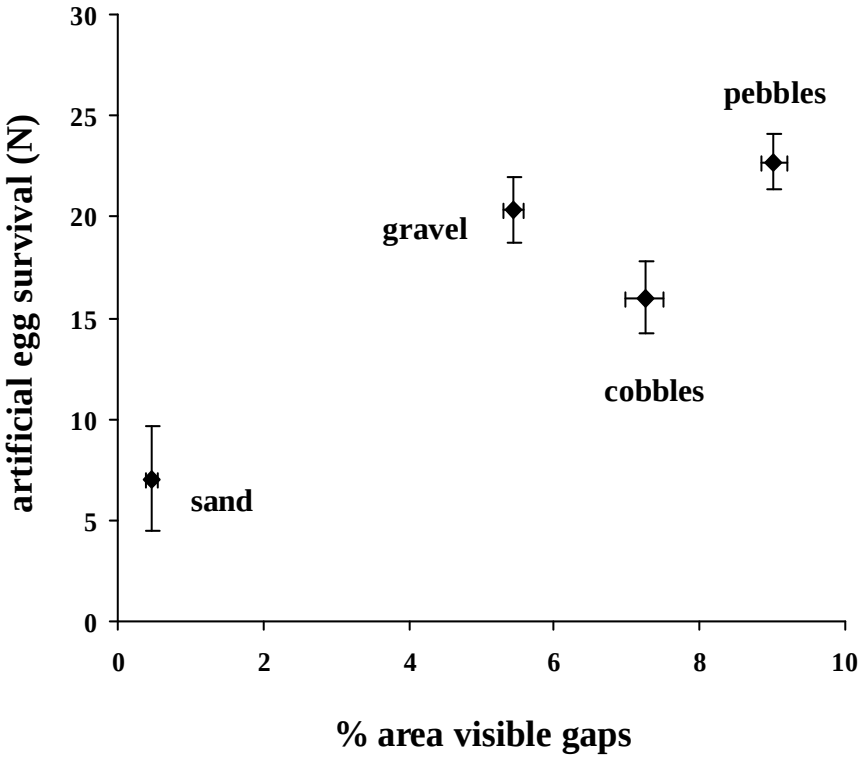


Figure 6.3 Mean (± S.E.) percentage area of visible gaps between sediment particles of sand, gravel, pebbles and cobbles and mean (± S.E.) number of eggs removed from tank of different sediments

Table 6.1 Summary of transects completed in Loch Lomond (A = Ross Bay beach, B = right of Ross Bay beach, C = left of Ross Bay beach), Loch Eck, Loch Sloy and Carron Valley Reservoir

	Lomond A	Lomond B	Lomond C	Eck	Sloy	Carron Valley
NGR	NS 368 955	NS 369 954	NS 367 955	NS 140 946	NN 284 113	NS 715 839
N transects	10	4	2	4	2	3
Between transects (m)	10	20	20	20	20	20
Mean transect distance (m)	71.7	101.3	57.5	30.5	33	43
N point surveys	239	81	25	40	22	42
Approx. area covered (m2)	6453	6075	1150	915	330	886
Mean depth	2.5	5.4	4.0	2.1	3.1	2.6
Max depth (m)	5.0	11.5	9.9	6.0	6.3	4.5
Commonest material	Macrophytes (44.9 %)	Sand (50.9 %)	Sand (46.0 %)	Sand (68.6 %)	Boulders (46.8 %)	Sand (41.2 %)

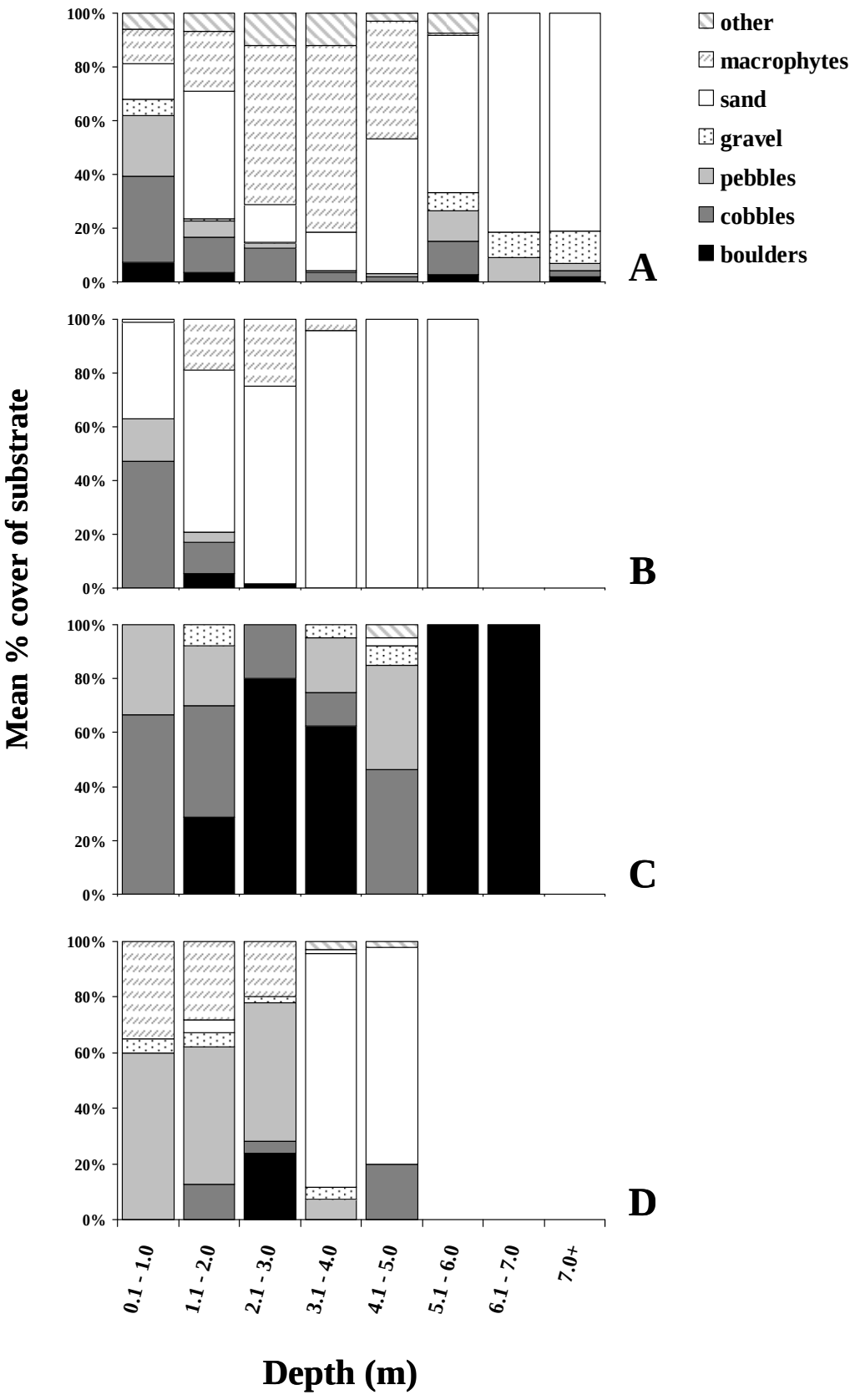


Figure 6.4 Mean percentage cover of sediment, macrophytes and other organic material found at different depths on point surveys in transects in Loch Lomond, Loch Eck, Loch Sloy and Carron Valley Reservoir

6.4.3 Estimated predation risk on spawning grounds

The substrates found in the transects were sand, gravel, pebbles, cobbles and boulders, however, organic material such as macrophytes and organic detritus (i.e. dead leaves, wood) were also found as part of the percentage cover (Fig. 6.4). The equation describing the relationship between substrate size and artificial egg survival was used to calculate a possible predation risk for larger particle sizes found on the surveying that could not be tested in the laboratory i.e. boulders. Extrapolated egg survival was 12.9 for small boulders and (at 256 mm, minimum size of boulders). The experimental data of mean artificial egg survival and surveys of the substrate composition on whitefish spawning grounds at four sites allowed the calculation of an index of relative predation risk on whitefish eggs by ruffe at these sites. The highest predation risk was found to be on areas of 100% sand, this was considered to be very high risk. Other groups were derived by dividing the remaining risk into three equally wide categories. The point surveys were then partitioned according to predation risk into low (0.24 – 0.41), medium (0.41 – 0.58), high (0.58 – 0.75) and very high (0.75+) predation risk categories. It was found that sequentially the highest overall predation risk (mean ± se) was found at Loch Eck 0.70 ± 0.02 (high risk), Loch Lomond 0.66 ± 0.01 (high risk), Carron Valley Reservoir 0.53 ± 0.04 (medium risk) and Loch Sloy 0.46 ± 0.02 (medium risk).

6.5 DISCUSSION

Invasive ruffe are important predators of whitefish eggs at spawning time in Loch Lomond, and have been found in large numbers on whitefish spawning grounds (Etheridge, 2009). Adams & Tippett (1991) demonstrated that ruffe consume relatively large quantities of whitefish eggs compared to other native fish at this site. Whitefish are broadcast spawners which utilise well-washed gravel of littoral areas or offshore banks of standing waters (Slack *et al.*, 1957). However, unlike many other salmonids (e.g. Sargent *et al.*, 1987), whitefish do not build a nest or redd to provide protection for their eggs. Therefore, their eggs are highly vulnerable with the substrate on the spawning grounds providing the only physical protection.

Whitefish eggs have a high natural mortality due to a range of physical and biotic factors. It was estimated by Slack *et al.* (1957) that in Loch Lomond, 65 % of eggs are predated on by trichoptera larvae and 30 % are either infertile or die from fungal attack. The eggs are also predated on by native fish, including powan (Slack *et al.*, 1957; Adams & Tippett,

1991). Brown *et al.* (1991) compiled a life table for Loch Lomond whitefish that found that mortality at the egg stage is 99.95 %. Thus egg survival is clearly a bottleneck for recruitment. If egg predation by ruffe is additional to these sources, this might be very important as a population regulation mechanism. Certainly, Brown and Scott (1994) concluded that the Loch Lomond whitefish population relies on the regular recruitment of large year classes, a few years without which may cause significant problems in terms of population viability.

The protective ability of different substrates commonly found on whitefish spawning grounds was assessed in ruffe foraging experiments on egg substitutes over these substrates. It has been shown that substrate type is important to egg survivorship and therefore has the potential to affect whitefish recruitment and population size. Pebbles offer the best protection for eggs from ruffe predation. It was found that protection of artificial eggs from ruffe predation over pebbles is 3.2 times greater than over sand, and is 1.4 times greater than over cobbles. There was a general increase in the protective ability of substrate with the area of visible gaps. However, the total area of visible gaps between substrate particles cannot be equated with the interstitial space, which is well known to increase with substrate particle size.

It was found during observation that the ruffe very quickly located and consumed the majority of the artificial eggs exposed on sand. In the larger particle substrates of gravel and pebbles, if the artificial eggs fell into a visible gap, they were protected from predation. However, it was found that the gravel particles were sometimes moved allowing access to the artificial eggs, while, the larger and deeper gaps in pebbles seemed to result in slightly better protection. On cobbles artificial eggs that fell on the surface of the cobbles were exposed to predation. While the visible gaps did provide some protection the gaps between cobbles were large enough that the ruffe could often enter them to forage. This effect of large gaps on foraging efficiency has been reported in other studies of fish predation on salmonid eggs (Biga *et al.*, 1998; Chotkowski & Marsden, 1999). This factor explains why cobbles had a low artificial egg survival compared to the area of total visible gaps. It was also observed during experiments that is was most often the largest fish which moved onto the feeding tray first and most often displayed aggressive behaviour to the other fish. However, the largest fish was not always able to enter these gaps, and the smaller fish could be at an advantage on cobbles.

The experimental results may also reflect protection of eggs from other fish predators and physical damage such as being swept off spawning grounds. However, invertebrate predation appears to be a significant source of whitefish egg mortality in some areas (Slack, 1955; Fox, 1978). It is possible that the substrates that provide the best refuges for eggs against fish predation may also protect these invertebrates. This would be part of the natural mortality of eggs previously measured, and which whitefish have sustained, rather than the additional mortality of invasive ruffe. Nevertheless, this and the interaction of ruffe-size in accessing certain sizes of visible gap may modify the most ideal (protective) size of whitefish spawning substrate in different sites.

In Scotland, spawning areas in two successful refuge sites show higher levels of protection from ruffe predation than spawning areas in native population sites. The results thus suggest that the refuge populations of whitefish in Scotland might be better able to withstand an invasion of ruffe than the native populations. Ruffe are likely to invade Loch Sloy in the future due to a planned pump-storage addition to the Loch Sloy hydroelectric scheme that will soon bring water from Loch Lomond (already invaded by ruffe) to Loch Sloy.

Ruffe are now well established in Loch Lomond and it is highly doubtful that they will ever be removed or controlled at a low population number successfully. While the addition of pebbles of spawning grounds with a deficit of ‘ideal’ substrate is a possibility, though a potentially expensive one, it remains to be seen how the whitefish population will respond to the increased predation mortality at this life stage in the longer term. There is a long history of introduction of non-native species in Britain, both by accident and design (Manchester & Bullock, 2000; Copp *et al.*, 2005). It is unfortunate that the long-term ecological impact of these introductions has gone largely unexamined, and studies which provide an insight into firstly adaptation in an invading species, and secondly in affected native species are almost absent. There are examples of situations whereby life-history responses, such as egg incubation and emergence timing responding to high mortality at the egg stage (Wedekind, 2002; Jones *et al.*, 2003; Kusch & Chivers, 2004; Wedekind & Muller, 2005). It remains to be seen if whitefish could respond to, this increased mortality due to predation at the egg stage.

It is apparent that egg incubation is an important bottleneck to recruitment to whitefish. This study indicates that spawning site substrate is critical for the survival of whitefish eggs from predation. This has significant management implications for spawning areas of

rare whitefish where additional mortality at this life-stage has potentially serious implications for population viability.

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Chapter 7 CONTINUOUS VARIATION IN THE PATTERN OF MARINE VERSUS FRESHWATER FORAGING IN BROWN TROUT FROM LOCH LOMOND, SCOTLAND[3]

7.1 ABSTRACT

The brown trout (*Salmo trutta*) is conventionally regarded as exhibiting a binary migration pattern, with individuals either adopting 1) residency, or 2) anadromy. Using carbon stable isotope analysis to distinguish between marine and freshwater sources of assimilated carbon, it is shown that some individual trout appear to follow an intermediate strategy of either, repeated movement between fresh water and marine, or estuarine residency. Carbon stable isotope ($\delta^{13}C$) values from Loch Lomond trout muscle tissue ranged from those indicative of assimilation of purely freshwater-derived carbon to those reflecting significant utilisation of marine-derived carbon. A single isotope, two source mixing model indicated that, on average, marine C made a 33 % contribution to the muscle tissue C of Loch Lomond trout. $\delta^{15}N$ but not $\delta^{13}C$ was correlated with fork length suggesting that larger fish were feeding at a higher trophic level but that marine feeding was not indicated by larger body size. These results are discussed with reference to migration patterns in other species.

7.2 INTRODUCTION

The brown trout (*Salmo trutta* L.) is a highly polytypic salmonid, showing phenotypic variation between and within populations to the extent that in the past these have been considered separate species similarly to other similar species (see Chapter 1). Individual fish within a population show considerable variation in life history characteristics and are facultatively anadromous (Elliott, 1994; Klemetsen *et al*, 2003; Cucherousset *et al*, 2005). Spawning occurs in natal streams; in open systems, a variable proportion of the total population, but rarely the whole population, undergoes a metamorphosis (smolting), that adapt individuals to life in salt water and these fish subsequently migrate to sea to feed. Other individuals within the population remain in fresh water as residents (McDowell, 1988; Jonsson & Jonsson, 1993; Elliott, 1994). Therefore, a binary choice of life history trajectories for individuals is conventionally described in *S. trutta* (Eek & Bohlin, 1997; Bagliniere *et al*, 2001; Charles *et al*, 2004); namely 1) freshwater residency, or 2) anadromy - migration to sea to feed before returning to natal streams to spawn.

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Anadromy in salmonids is well studied, particularly in Atlantic salmon (*Salmo salar* L.) (McDowell, 1988; Fleming, 1996; Garcia-Vazquez *et al*, 2001). The benefits of migration to sea include access to more profitable food resources and so increased growth (Berg & Jonsson, 1990; Olsson *et al*, 2006), while the costs include increased predation by marine predators and an energetically costly migration (Bohlin *et al*, 2001; Dieperink *et al*, 2002). Females have a significantly higher energy requirement than males and thus are more likely to adopt an anadromous pathway (Elliott, 1994; Klemetsen *et al*, 2003). The benefits of anadromy are thus less obvious for males. Large anadromous males compete for females directly, while small resident males can adopt 'sneaking' reproductive tactics in which they do not compete directly for access to the female. These 'sneaking' males can have substantial reproductive success, without the costs of migration (Myers & Hutchings, 1987; Garcia-Vazquez *et al*, 2001). However, despite the life history differences between them, migrant and resident trout can spawn together successfully. The prevalent view, therefore, is that where resident and anadromous trout spawn or occur together they are freely interbreeding fractions of a single spawning stock (Elliott, 1994; Klemetsen *et al*, 2003).

A number of laboratory techniques have been used to identify resident and anadromous fish, including: carotenoid pigment profiling (Youngson *et al*, 1997), measurement of the strontium content of scales and bony tissue (Kalish, 1990; Eek & Bohlin, 1997; Veinott *et al*, 1999) and analysis of stable isotope ratios (McCarthy & Waldron, 2000; Jardine *et al*, 2005). Stable isotopes of nitrogen and carbon are widely used in the study of animal movements and trophic interactions (Grey 2001; Grey *et al*, 2002; Harrod *et al*, 2005). Naturally occurring stable isotopes are assimilated by animals and fractionation by biochemical processes causes the heavier isotope to be accumulated in animal tissue. $\delta^{15}N$ (the change in the ratio of ^{15}N to ^{14}N compared with a standard) is typically enriched by ca. 3-5‰, allowing the long-term trophic position of consumers to be estimated (Peterson & Fry, 1987; Post, 2002; Sweeting *et al*, 2007). In contrast, trophic enrichment in $\delta^{13}C$ (the change in the ratio of ^{13}C to ^{12}C compared with a standard) is typically minor (ca. <1‰) and $\delta^{13}C$ is used as a robust and consistent indicator of the carbon source the organism has been assimilating (Peterson & Fry, 1987; Hobson, 1999). $\delta^{13}C$ has specifically been used to distinguish between fresh water and marine carbon sources in a range of mobile consumers in a number of studies (Hobson, 1999; Harrod *et al*, 2005; Guelinckx *et al*, 2006). $\delta^{13}C$ values are relatively ^{13}C depleted (more negative) in freshwater compared to marine habitats, with a gradient between the two extremes that correlates with salinity (Fry & Sherr, 1984; Riera & Richard, 1996).

Different tissues vary in the rate at which their isotopic values reflect that of their food source, with high turnover tissues such as liver changing quickly and thus responding to a change in diet rapidly, while low turnover tissues such as bone changes slowly (Bearhop *et al*, 2004). The turnover of muscle depends on growth rate, and falls somewhere in between these two extremes, being measured in months (Perga & Gerdeaux, 2005; Phillips & Eldridge, 2006).

A general anecdotal observation from earlier studies of fish in Loch Lomond has been that many of the trout caught in Loch Lomond were small in size, consistent with fresh water residency, but also silver in colouration consistent with anadromy. Here, analysis of the stable isotopes of carbon and nitrogen in muscle tissue is used to test the null hypothesis that *Salmo trutta* exhibit a typical binary sea migration pattern in Loch Lomond, Scotland.

7.3 METHODS

In total 75 multi-panel Nordic-pattern gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set overnight in sites in the north, mid and south basins of Loch Lomond (see Appendix 1) over the winter of 2005/06 (from 9 November 2005 to 24 January 2006) as part of a broader fish survey. These nets are non-selective for salmonids within the modal size range 45 to 495 mm fork-length (Jensen & Hesthagen, 1996). During this period, 44 brown trout were caught. Fish were frozen within 4 hours of capture. In the laboratory, fish were defrosted, scales were removed below the dorsal fin for aging, and a small piece of white muscle posterior to the head and above the lateral line was removed for stable isotope analysis. Tissue was dried at constant temperature (50 °C for at least 48 hours), ground to a fine powder using a grinder (Revel Ltd.) and 0.5 mg of dried ground muscle was packed into pressed 10x10 mm tin weighing pans and used in simultaneous analysis of stable C and N isotopes. Stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry at the Max Planck Institute for Limnology, Germany. Stable isotope ratios are given using the δ notation expressed in units per mil where δ (‰) = [(R sample/R standard)-1] x 1000, and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The reference materials used were secondary standards of known relation to the international standards of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Typical precision for a single analysis was ± 0.1 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. All $\delta^{13}C$ values were subsequently adjusted for lipid concentration variation (Kiljunen *et al*, 2006).

To determine the relative contribution of energy derived from fresh water and marine sources, a single isotope, two source mixing model was applied to the trout $\delta^{13}C$ data (Phillips & Gregg, 2001). This approach assumes that marine and fresh water represent the only two sources of available carbon, and that the $\delta^{13}C$ values of the tissue are representative of the diet of the fish. It was also assumed that the trout with the most depleted $\delta^{13}C$ values represented individuals assimilating only freshwater derived carbon, *i.e.* 100 % fresh water feeding, and the trout with most enriched $\delta^{13}C$ values were wholly deriving their energy from marine sources *i.e.* 100 % marine feeding. A mean freshwater $\delta^{13}C$ value was also derived for two freshwater fish species from Loch Lomond, namely bream (*Abramis brama* L.) and powan (*Coregonus lavaretus* L.). A cross-species mean marine $\delta^{13}C$ value for 11 North East Atlantic species was also derived from the literature. Species included were albacore tuna *Thunnus alalunga* (Bonnaterre), whiting *Merlangius merlangus* L., flounder *Platichthys flesus* L., monkfish *Lophius budegassa* (Spinola), hake *Merluccius merluccius* L., red mullet *Mullus barbatus* L., tope *Galeorhinus galeus* L., black-mouth catshark *Galeus melastomus* (Rafinesque), starry smooth hound *Mustelus asterias* (Cloquet), spiny dogfish *Squalus acanthias* L. and lesser-spotted dogfish *Scyliorhinus canicula* L. (Das *et al*, 2000; Pinnegar *et al*, 2001; Badalamenti *et al*, 2002; Domi *et al*, 2005).

7.4 RESULTS

Of the 44 trout sampled in winter 2005/06 from fresh water in Loch Lomond, muscle tissue $\delta^{13}C$ values ranged between -27.7 ‰ and -17.8 ‰. The most depleted (negative) $\delta^{13}C$ values recorded were consistent with those derived from obligate freshwater fishes (powan and bream), -27.2 ± 0.9 (mean ± S.E.). At the other extreme the most enriched (positive) $\delta^{13}C$ values from trout were consistent with mean (± S.E.) values calculated from North East Atlantic marine fishes (-17.8 ± 0.3). Between these extremes, the trout showed a wide range of $\delta^{13}C$ values (Fig. 7.1). The nitrogen isotope analysis of trout tissue samples show signatures ranging from 8.4 ‰ to 14.4 ‰. There was a weak, but statistically significant positive relationship between $\delta^{13}C$ and $\delta^{15}N$ ($F_{1,43} = 4.43$, $R^2_{(adj)} = 0.07$, $P < 0.05$).

A two source linear mixing model was used to determine the contribution of freshwater and marine carbon sources to trout muscle tissue collected in Loch Lomond at this time using the $\delta^{13}C$ values adjusted for lipid variation. Fig. 7.2. shows the relative frequency distribution of calculated marine source C contribution in muscle tissue. The modal marine

contribution to muscle tissue from this sample was 30 %, with the lower and upper and lower interquartiles being 19 % and 42 % respectively.

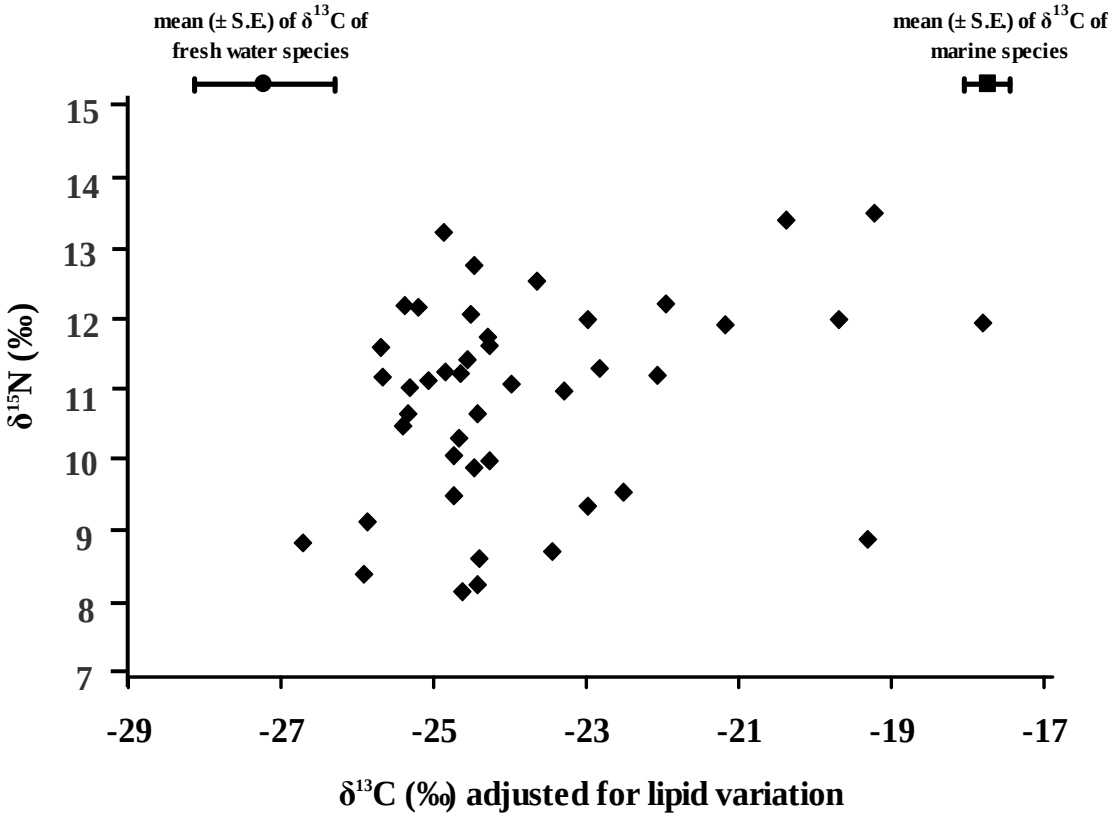

Figure 7.1 $\delta^{13}C$ adjusted for lipid variation and $\delta^{15}N$ of muscle from brown trout from Loch Lomond. Mean fresh water $\delta^{13}C$ for non-migratory fish from Loch Lomond -27.2 ± 0.9 (mean ± S.E.) is shown by ●, and mean marine $\delta^{13}C$ for North East Atlantic species -17.8 ± 0.3 (mean ± S.E.) is shown by ■ (Das *et al.*, 2000; Pinnegar *et al.*, 2001; Badalamenti *et al.*, 2002; Domi *et al.*, 2005).

Regression analysis was used to explore any factors that might be affecting the stable isotope results. Date of capture was not correlated with $\delta^{13}C$ ($F_{1,43}$=0.55, $R^{2}_{(adj)}$=-0.01, P=0.46) or with $\delta^{15}N$ ($F_{1,43}$ = 0.12, $R^{2}_{(adj)}$ = -0.02, P = 0.73). Log_e L_f was not correlated with $\delta^{13}C$ ($F_{1,42}$ = 1.8, $R^{2}_{(adj)}$ = 0.02, P = 0.19), however, there was a significant correlation between L_f and $\delta^{15}N$ of Loch Lomond trout (Fig. 7.3.) ($F_{1,42}$ = 37.6, $R^{2}_{(adj)}$ = 0.47, P < 0.001). The L_f and age are not affected by the $\delta^{13}C$ signature of Loch Lomond trout muscle tissue (MANCOVA, $F_{1,42}$ = 1.1, p = 0.34).

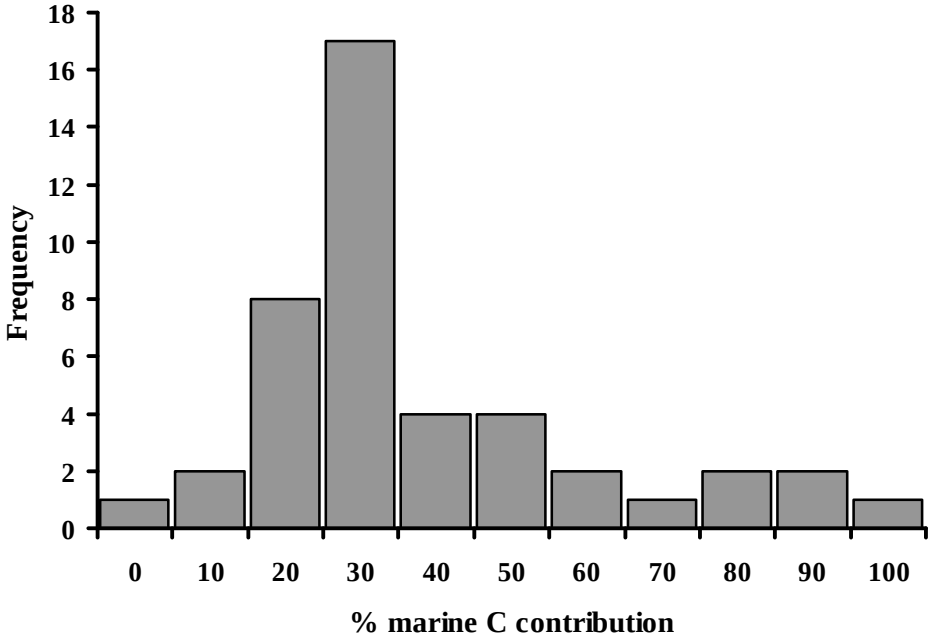

Figure 7.2 The percentage estimated marine C contribution to tissue of brown trout from Loch Lomond calculated using a single isotope, two source linear mixing model applied to the data (Phillips & Gregg, 2001). The simple mixing model assumes that brown trout with the most depleted $\delta^{13}C$ value (-27.7 ‰) represents a wholly fresh water foraging history, and the brown trout with most enriched $\delta^{13}C$ value (-17.8 ‰.) represents a wholly marine foraging history

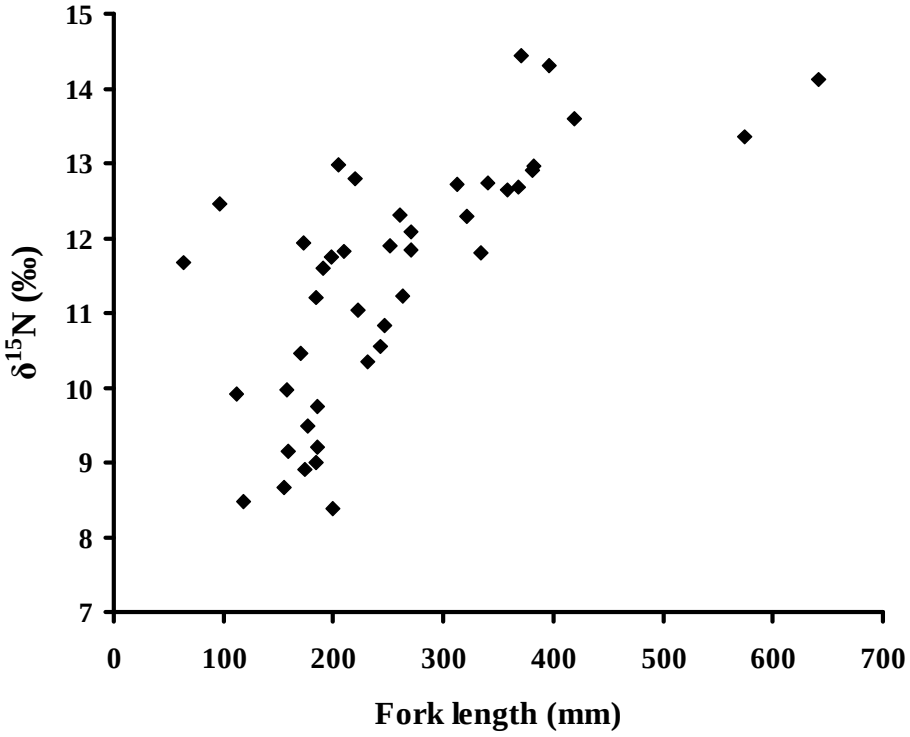

Figure 7.3 Fork length and $\delta^{15}N$ of muscle from brown trout from Loch Lomond. There is a significant positive correlation ($F_{1,42} = 37.6$, $R^2(adj) = 0.47$, $p < 0.001$).

7.5 DISCUSSION

Stable isotope analysis of muscle tissue of trout caught in Loch Lomond revealed an unexpectedly broad range of values for both $\delta^{13}C$ and $\delta^{15}N$. The range of $\delta^{13}C$ is consistent with the trout population in Loch Lomond feeding and assimilating C from both freshwater and marine systems. The distribution of $\delta^{13}C$ values is not however consistent with a dichotomous marine/fresh water foraging strategy, where anadromous fish migrate to sea to feed and residents remain in fresh water feeding. Rather these data show more continuous variation between freshwater and sea feeding (and consequently migration extremes) in this population.

Both $\delta^{13}C$ and $\delta^{15}N$ are enriched in marine environments in comparison to fresh water (Mizutani & Wada, 1988; Post, 2002), and this is supported in the positive relationship between $\delta^{13}C$ and $\delta^{15}N$ from trout in this study. The strong positive relationship between L_f and $\delta^{15}N$ (Fig. 7.3.) is indicative of larger fish feeding at higher trophic levels than smaller individuals. The individual with the lowest $\delta^{15}N$ value was smaller (200 mm) and had a depleted $\delta^{13}C$ value (-24.6 ‰) indicating freshwater residency, in comparison to the individual with the greatest $\delta^{15}N$ value which was larger (371 mm) and had an enriched $\delta^{13}C$ value (-19.2 ‰) indicating a more marine signature. Due to the weak relationship between $\delta^{13}C$ and $\delta^{15}N$, the variation in $\delta^{15}N$ is likely due to larger trout feeding at a higher trophic level, rather than differences in the baseline $\delta^{15}N$ values between freshwater and marine habitats. The MANCOVA results indicate that growth does not appear to be significantly affected by $\delta^{13}C$ value, which suggests there is no growth benefit in migration for these trout. However, this also reflects that by chance, no larger and older fish were caught that had a large proportion of marine C contributing to muscle tissue; alternatively, it is possible that these fish may over winter in the marine environment (Olsen *et al.*, 2006).

The results of the linear mixing model show frequencies of estimated marine C contribution to muscle $\delta^{13}C$ that are consistent with individuals having variable proportions of the assimilated diet coming from fresh water and saltwater sources. The mean foraging strategy of the trout sampled here suggests that 33 % of muscle tissue in winter is derived from marine sources. The most likely explanation is that many trout in this population either spend most of their time in fresh water but move into seawater for a short period in the months previous to capture, or they spend a significant proportion of their time in an

intermediate area between fresh water and oceanic saltwater. There are three alternative, but less probable scenarios. One is that the diet of large resident trout is partially reliant on small anadromous fish, resulting in partially marine isotope values. However, there is no significant size difference between trout with a more marine $\delta^{13}C$ values and those with a more fresh water $\delta^{13}C$ values suggesting predation would be problematic, and trout with intermediate isotope values are not noticeably larger. The second is that resident trout have been feeding primarily on $\delta^{13}C$ enriched invertebrate prey such as freshwater snails or other epilithic microalgal scrapers (Finlay *et al*, 1999). Since trout are have been shown to be opportunistic feeders it seems unlikely they would specialise enough on prey species of the same trophic guild to affect the diet to such an extent. The third is that anadromous trout are feeding in fresh water on their return migration and so are diluting the marine signature of their muscle tissue. If this were the case a correlation between date of capture and marine C signal would be expected, however there was no evidence for such a relationship. Furthermore, depressed feeding and growth in late autumn and winter leads to slow tissue turnover rates in winter, and the bulk of the carbon assimilated into muscle tissues reflects summer feeding (Perga, & Gerdeaux, 2005).

Movement at sea in *S. trutta* is known to be more geographically restricted and shorter in duration than *S. salar* (McDowell, 1988). Sub-categories within resident and anadromous trout groups have been previously described. Resident trout may 1a) spend their entire life in their natal stream; 1b) migrate from their natal stream to the parent river, 1c) migrate from their natal streams to a lake; anadromous trout can be sub-categorised into 2a) short distance migrants (estuarine or slob trout) that migrate into estuaries to feed, 2b) long distance migrants that migrate to coastal waters (Elliott, 1994). Migration can also be restricted in time. Most anadromous trout spend at least 18 months at sea, but some returning fish spend only ca. 6 months away from fresh water, these individuals are commonly referred to as ‘finnock’ in Scotland. The proportion of finnock among returning fish varies considerably between rivers and years. In many populations most finnock are males, since these are small they follow a sneaking reproductive strategy (Elliott, 1994).

It is likely that there are benefits to following a life history intermediate between that of fully resident or anadromous fish. Estuaries are often used as nurseries by juvenile marine fish (Brown, 2006; Guelinckx *et al*, 2006) hence the most productive feeding areas for these trout may be in estuarine areas and not the sea (Elliott, 1986). It is also possible that some marine predators are avoided if the trout do not move into coastal waters. Other advantages of migration may include avoidance of intraspecific competition (Bult, 1999;

Landergren, 2004; Olsson *et al*, 2006). It is possible that Loch Lomond trout move only far enough towards sea to take advantage of the benefits without undertaking a strenuous and potentially hazardous migration to fully marine habitats.

Individual life history variation have been identified using stable isotope analysis in a number of species: white-spotted charr (*Salvelinus leucomaenis* Pallas) (Arai & Morita, 2005), pond smelt (*Hypomesus nipponensis* McAllister) (Arai *et al.*, 2006), European eel (*Anguilla anguilla* L.) (Harrod *et al.*, 2005), and Japanese eel (*Anguilla japonica* Temminck & Schlegel) (Tzeng *et al.*, 2002, 2003). Moreover, phenotypic plasticity is well known in many other species using other methodologies as explored between powan populations in Chapter 3 and 4. However, this is the first time stable isotope analysis has been used to elucidate complex migration of individuals in a trout population. These findings suggest that Loch Lomond trout have a flexible migration strategy with a high degree of behavioural plasticity with an ability to utilise the full range of salinities available. This pattern is examined in the context of other Loch Lomond fish species in Appendix 3.

Chapter 8 GENERAL DISCUSSION

In this thesis six studies (Chapters 2 to 7) are presented that have implications for management of UK *Coregonus spp.* and for management specifically of Loch Lomond as a site of significant biological interest.

8.1 PUTATIVE SPECIES IN BRITISH WHITEFISH

As discussed in Chapter 2, a name change although perhaps seemingly superficial, can have far-reaching implications for conservation and dissemination of information between scientists, and between scientists and the wider community. If a population is designated a different species, any legal protection the population enjoyed prior to the change must be transferred. However, as discussed in Chapter 1, the designation and naming of species can be problematic, what is being described is essentially a moment in time of a dynamic process (speciation). Phenotypic differences between populations of phenotypically variable and plastic organisms may not necessarily denote species differences. Here (Chapter 2) it was demonstrated that there was insufficient evidence to warrant the splitting of *Coregonus lavaretus* populations into three species endemic to Britain. That this splitting appears to have been accepted without question by some authorities is worrying. It is recommended that populations of British whitefish are retained as *C. lavaretus*, until other, statistically robust, evidence is presented.

Other UK and Irish whitefish species have also been designated endemic species by Kottelat & Freyhof (2007) from their currently recognised pan-continental species designations, vendace (*C. albula* to *C. vandesius*) and pollan (*C. autumnalis* to *C. pollan*). It is urged that these and other new designations of European freshwater fishes (e.g. Arctic charr) are examined critically before acceptance.

8.2 INTRA-SPECIES DIFFERENCES IN *C. LAVARETUS* POPULATIONS

However, it is apparent in Chapter 2 that there are differences between UK *C. lavaretus* populations. Comparative investigations have been carried out on the two native populations of *C. lavaretus* (powan) in Scotland, in previous studies (Brown & Scott, 1990; Pomeroy, 1991; Brown & Scott, 1994; Dolezel & Crompton, 2000) and in this thesis (Chapter 3). While there are many similarities between the sites, it was demonstrated in this study between Loch Lomond and Loch Eck powan that there were indeed significant differences in morphology and ecology between these two populations. In addition, provisional stable isotope analysis (SIA) results in Appendix 4 of significant differences in

both $\delta^{13}C$ and $\delta^{15}N$ values have indicated long term differences between diet in these two populations. Loch Lomond powan appeared to eat a very similar diet to each other, indicated in tightly bunched $\delta^{13}C$ values of individuals, while Loch Eck powan individually appeared to have a more varied diet, obtaining their C from more than one source. This is supported by previous work on diet differences by Pomeroy (1991) which demonstrated that Loch Lomond powan feed primarily on plankton while Loch Eck fish feed on both plankton and benthos. Due to difficulties in obtaining consistent baseline indicators, analysis of summer and winter stomach contents would provide further grounding for this information in the future. In order to quantify the uniqueness of British *C. lavaretus* populations in the context of morphology, ecology, life history and genetics, comparisons of these should be extended to cover all populations. Until alternative evidence is presented all British *C. lavaretus* populations must be (or continue to be) managed as evolutionary significant units (ESUs).

8.3 CONSERVATION OF UK WHITEFISH

Important population differences found between populations that are potentially vulnerable to impacts such as invasive species and eutrophication may require conservation action to be initiated. The translocation of individuals to a new site has become a popular conservation measure for whitefish (Winfield *et al.*, 2002, 2008c). In Chapter 4 it was demonstrated that morphological and ecological differences can develop quickly after conservation translocation to a novel environment. Significant differences were found, not only between the donor and refuge populations, but between the refuge populations, highlighting the effect that environmental differences can have on phenotypically plastic species. Other differences were indicated by differences in preliminary SIA results recorded in Appendix 4 which indicated significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values of powan in all pairwise comparisons apart from $\delta^{13}C$ values between Loch Lomond and Loch Sloy powan. Loch Sloy powan appeared to have a very different long term diet, feeding almost one trophic level, below fish from any other site indicated by low $\delta^{15}N$ values, and individuals appeared to obtain their C from very wide ranging sources. The differences between these populations were reflected by the cluster analysis in Chapter 2 in which Loch Sloy powan did not cluster as closely with Loch Lomond powan as those from Carron Valley Reservoir. Thus, these results emphasise the fact that fish introduced from a one site to another may not in effect remain the same fish. Nevertheless, it is thought that the differences between the populations were mostly due to plasticity, though in the much longer term further differences (including genetic differences) could develop. Translocation to a new site should be used as a conservation measure for isolated

populations of high conservation value phenotypically plastic species. However, this should never be used in preference to (or to the exclusion of) *in situ* conservation measures where these are possible.

8.4 LOCH LOMOND

Another important aspect of conservation translocation is the investigation of sub-structuring within populations. Translocations need to encompass as much genetic diversity as possible, while, for *in situ* conservation, the differing needs of possible sub-populations must be taken into account, for instance protection of certain spawning grounds, or discrete food and habitat resources. It has become apparent in the course of this thesis that Loch Lomond is a site of significant biological interest, in particular the feeding ecology of fish at this site. While there are no sub-populations within Loch Lomond powan, as demonstrated in Chapter 5 despite the inter-basin differences described in Appendices 1 and 2, there does appear to be feeding site fidelity in Loch Lomond powan. A freshwater feeding morph of the river lamprey (*Lampetra fluviatilis*) is found in Loch Lomond and appears to feed extensively on powan (Maitland, 1980; Adams *et al.*, 2008) (Appendix 3). How the potential decline of Loch Lomond powan (Chapter 3) will affect interactions such as this is not clear, but a decline in this rare river lamprey morph has been indicated by Adams *et al.* (2008). Brown trout (*Salmo trutta*) in Loch Lomond also appear to have an interesting trophic ecology. Rather than having a resident or anadromous life choice, some trout appear to spend either small amounts of time at sea, or migrate only part of the way to sea as indicated by intermediate stable isotope values of the muscle of some individuals between purely freshwater and purely marine $\delta^{13}C$ values (Chapter 7). This demonstrates the phenotypic plasticity in other fish species that must be taken into account in ecological and conservation studies. There has also been a large effect by invasive ruffe (*Gymnocephalus cernuus*) on the trophic ecology of Loch Lomond (Adams, 1991; Adams & Mitchell, 1995; McCafferty, 2005). Ruffe have a very varied diet indicated by the large range of values for $\delta^{13}C$ indicating C source and $\delta^{15}N$ indicating trophic level for long term feeding of individual ruffe (Appendix 3). They thus form a complex and non-discrete part of the food web of Loch Lomond. As discussed (Chapter 1), interactions between species such as predation and competition can have large, multidirectional and probably unpredictable impacts as part of a heterogeneous and non-static environment.

8.5 INVASIVE SPECIES: POSSIBLE IMPACTS AND MITIGATION

Ruffe directly impact powan through egg predation, which in Loch Lomond has previously been demonstrated by Adams & Tippett (1991) as a significant and recent ecological

pressure on this species. Whitefish populations in the UK are affected by invasive species (e.g. Winfield *et al.*, 2007b), which have been attributed along with other factors as a reason for the extinction of vendace in Scotland and the decline of other British and Irish *Coregonus spp.* (Maitland, 1966; Winfield *et al.*, 1996). The long-term effect of invasive species is of considerable interest, particularly as there are usually few avenues of mitigation once invasive species are established. In Loch Lomond, obvious evidence of an effect on the life history of powan from the introduction of ruffe has not yet been found. In Appendix 5, a life table constructed using information collected in this study differs very little from a previous life table constructed by Brown *et al.* (1991). However, the life table relied heavily on information from Brown *et al.* (1991) particularly for earlier life stages. The impacts of ruffe in increasing powan mortality might be responded to in egg size and number, and timing of spawning, or hatching. Certainly in other species there are examples of life history responses, such as egg incubation and emergence timing responding to high mortality, particularly in amphibians (Warkentin, 1995; Vonesh, 2005) and fish (Wedekind, 2002; Jones *et al.*, 2003; Kusch & Chivers, 2004; Wedekind & Muller, 2005; Evans *et al.*, 2007) The possible effect of this may be unpredictable and profound as changes in the life history at one life stage may have fitness consequences at another (Ojanguren *et al.*, 1996; Jones *et al.*, 2003; Kusch & Chivers, 2004). It is therefore imperative that further investigation into potential impacts of increased mortality at the eggs stage be carried out.

The study presented in Chapter 6 of this thesis investigated the foraging success of ruffe over different substrates found on powan spawning grounds. It was found that pebbles and gravel were the best spawning substrate to protect eggs from ruffe foraging. Although this does not include any other mortality, such as that due to invertebrate predation, this information might be used to assess the potential value spawning grounds in native sites. It may also be used to assess the extent of good spawning grounds in potential translocation sites. Speculatively, there may also be the potential for improvement of spawning sites by the addition of better substrate for egg protection. However, the expense and difficulty of this may make such an intervention impractical.

8.6 RECOMMENDATIONS FOR MANAGEMENT AND FUTURE WORK

In conclusion, the studies presented in this thesis have resulted in several recommendations for conservation and management of UK whitefish, and for potential avenues of future research:

1) The populations examined here should remain as *C. lavaretus*. However, additional and more detailed studies in taxonomy and genetics of UK *Coregonus spp.* and their relationship with populations elsewhere are called for.

2) All populations should be treated (or continue to be treated) as ESUs.
   a) Comparative studies in order to quantify differences in morphology, life-history, ecology and genetics between populations should be undertaken.
   b) The possibility of sub-structuring in populations should be investigated.

3) Similar morphometric (and genetic) studies should be carried out on other British species, particularly those that show a high degree of phenotypic variation and plasticity.
   a) In particular this should include Arctic charr, also a UKBAP priority species which is highly variable between and within sites.
   b) This should include investigation into new species designations by Kottelat & Freyhof (2007).

4) Conservation action, particularly for those populations considered to be particularly unique, should be undertaken: this should include conservation translocation.
   a) Differences found between the native populations of Loch Lomond and Loch Eck indicate that at least one refuge population of Loch Eck powan should be established.
   b) Due to changes to the management of Loch Sloy (Appendix 1) (which may lead to the eventual invasion of ruffe into this site), at least one additional refuge population should be established from Loch Lomond powan.

5) Further work should include tracking phenotypic and genetic changes in new refuge populations to better understand the mechanisms of this change.

6) The possibility of laboratory based ‘common garden’ experiments should be explored to explore what variation is genetically based, and what is plastic, this may help to predict what short term phenotypic and ecological changes are likely in response to environmental changes.

7) The planned abstraction of Loch Lomond water to Loch Sloy will result in the opportunity for a large scale field experiment, this should not be ignored.

a) The time taken for ruffe to invade Loch Sloy may provide information useful to impact assessments of other such schemes.
b) This is also an opportunity to investigate the responses of an invader and native fish populations in a relatively simple ecosystem.

8) Further work on the potential for life history changes in Loch Lomond powan in response to an invasive species in comparison to previously collected data (e.g. Brown *et al.*, 1991) should be carried out.

9) Experiments on ruffe foraging on powan eggs should be extended to the field and should also involve investigation into the protective ability of macrophytes and the impact of other mortality factors.

Appendix 2 LOCATION AND DESCRIPTION OF STUDY SITES

There are seven native populations of *Coregonus lavaretus* in Britain. Two are located in Scotland, Four in England and one in Wales (Fig. A1.1.).

Figure A2.1 Locations in the UK of seven native populations of *C. lavaretus* in Scotland (A), England (B) and Wales (C).

The two native Scottish populations (locally known as powan) are found in Loch Lomond (56°05'N, 4°36'W) and Loch Eck (56°06'N, 4°59'W) which are in two different catchments. The two refuge populations were established using Loch Lomond powan in Loch Sloy (56°16'N, 4°47'W) and Carron Valley Reservoir (56°02'N, 4°06'W) and are

located within the Loch Lomond catchment (Fig. A1.2.). The four native English populations (locally known as schelly) are found in Brotherswater (54°30'N, 2°55'W) Haweswater (54°03'N, 2°48'W) Red Tarn (54°31'N, 3°31'W) and Ullswater (54°34'N, 2°54'W) (Fig. A1.3.). The single native Welsh population is found Llyn Tegid (52°54'N, 3°37'W) (Fig. A1.4.).

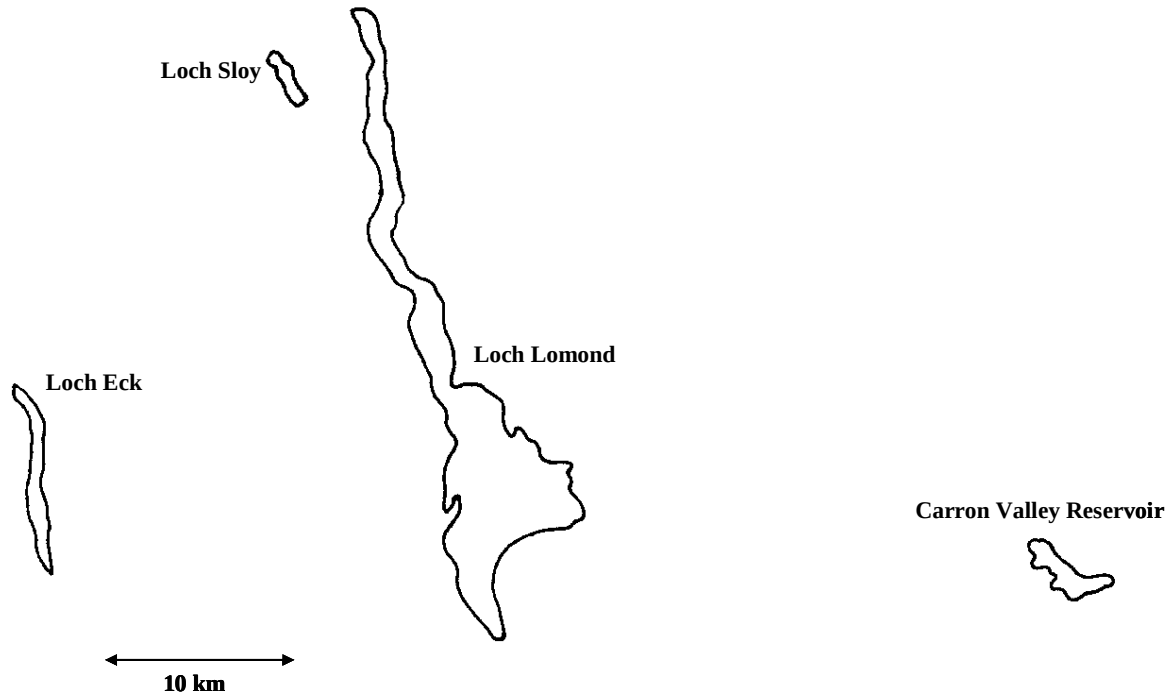

Figure A2.2 The sites sampled for *C. lavaretus* in this study: Scottish populations. Native: Loch Lomond and Loch Eck, refuge: Loch Sloy and Carron Valley Reservoir

A1.1 LOCH LOMOND

Loch Lomond is the largest single area of freshwater in Great Britain (71 km^2) and the third deepest (190 m) and is at an altitude of 7 m. The loch lies north to south along its long axis, with a narrow north and wide south. The geology of this area is responsible for the shape of the loch which was formed by glacial movement (Slack *et al.*, 1957; MacDonald, 1994). The glacier was constrained by hard metamorphic rocks in the north, forming a deep sided trough. Beyond Ross Point the loch widens, owing to a change to underlying softer sedimentary rocks. Hard pebbly grits form six islands, then the width of the loch increases to a maximum of about ca. 9 km, with a maximum depth of only 23m. The south is bisected by a chain of four islands on the line of the highland boundary fault (Slack *et al.*, 1957; MacDonald, 1994). The narrow deep north (highland-like) and wide shallow south (lowland-like) basins of Loch Lomond form a duel natured loch, which is

divided by an intermediate mid-basin. During the summer, thermal stratification occurs in the north, but in the south is transitory. The shallow phytal zone (< 4 m) makes up only about 10% of the total surface area of Loch Lomond, most of these shallows are in the south (Slack *et al.*, 1957; Mitchell, 2001). Due to the difference in geology, soils, population and land use between the north and south of Loch Lomond, the north is oligotrophic, while the south is more mesotrophic (Best & Traill, 1994; Mitchell, 2001). The divided nature of Loch Lomond may have an effect on where various species of fish in Loch Lomond are most likely to be found. Certainly in netting carried out to sample powan during spawning season on their spawning grounds captured a different array of fish in different areas. The three most common fish in all the basins were ruffe (*Gymnocephalus cernuus*), powan and roach (*Rutilus rutilus*) (Appendix 2). However, the north basin had relatively high numbers of pike (*Esox lucius*) and perch (*Perca fluviatilis*), perhaps suggesting that there was high levels of predation on adult powan. The mid basin had almost 1.5 times the number of ruffe as any other basin which suggests high mortality of powan eggs. The south basin had more roach than powan; this basin is more nutrient rich and so perhaps a better habitat for cyprinids in comparison to the other areas of Loch Lomond. Roach also probably compete with powan for food resources. The trophic interactions between species in Loch Lomond are unique and due to the species richness of this site, particularly interesting (Appendix 4). For instance a native species, the river lamprey (*Lampetra fluviatilis*), as a unusual resident feeding morph specialises on parasitising powan and in response powan appear to be able to sustain multiple attacks with little effect (Maitland, 1980; Adams *et al*,. 2008).

A1.2 LOCH SLOY

Loch Sloy is a small semi-natural loch to the north-west of Loch Lomond, it has a surface area of ca. 1 km^2, a maximum depth of ca. 40 m. Once a shallow loch, it was dammed as part of the Loch Sloy hydroelectric scheme between Ben Vorlich with Ben Vane. A pumped storage plan has recently received permission. This will involve the pumping up of Loch Lomond water into Loch Sloy and will likely result in the invasion of ruffe to this site.

A1.3 CARRON VALLEY RESERVOIR

Carron Valley Reservoir is located to the east of Loch Lomond; it has a surface area of ca. 3 km^2, and a maximum depth of ca. 10 m. Originally a brown trout (*Salmo trutta*) fishery, rainbow trout (*Oncorhynchus mykiss*) have recently been introduced as part of the fishery.

Thus far, the impact of this additional species on powan, for instance through predation is unknown.

A1.4 LOCH ECK

Loch Eck is a narrow loch surrounded by mountains, with a surface area of 4.6 km^2, a maximum depth of 42 m and is at an altitude of 9 m. It is in a different catchment to Loch Lomond, but is only 22km away and has similar geology and limnology (Brown & Scott, 1990) and is oligotrophic (Winfield *et al.,* 2009). Loch Eck is unique in being the only Scottish loch to have a salmonid fish fauna comprising Atlantic salmon (*Salmo salar*), sea and resident forms of brown trout, Arctic charr (*Salvelinus alpinus*) and powan. In Loch Eck, there has been much focus on the Arctic charr (Friend, 1955; Bush & Adams, 2007); it is though that there has been a large decline in this population (Winfield *et al.*, 2009), certainly only one specimen of Arctic charr was caught in nettings for this study (Appendix 2).

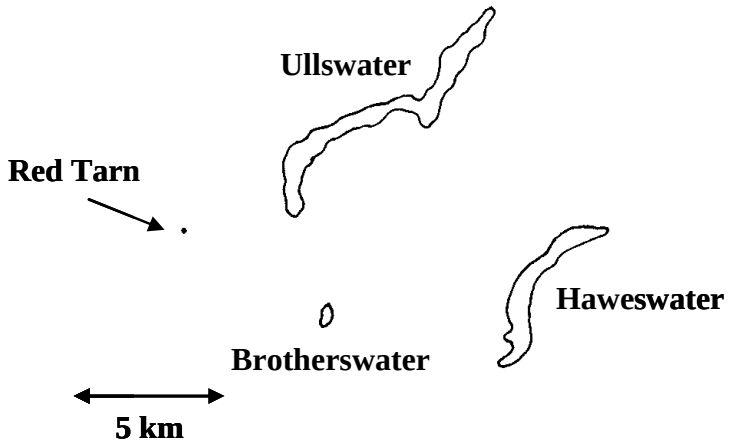

Figure A2.3 The sites sampled for *C. lavaretus* in this study: English native populations, Brotherswater, Haweswater, Red Tarn and Ullswater

A1.5 BROTHERSWATER

Brotherswater is a small lake with a surface area of 0.19 km^2, a maximum depth of 16 m, it is at an altitude of 173 m (Winfield *et al,*. 1993). It is located just to the south of Ullswater it is thought that these may have once been part of a much larger lake (Talbot & Whiteman, 2000). It is found, as most of the schelly lakes are, in Borrowdale volcanic rocks.

A1.6 HAWESWATER

Haweswater has a surface area of 3.9 km^2, a maximum depth of 57 m, it is at an altitude of 241 m (Bagenal, 1970). It is an oligotrophic semi-natural lake, a dam was built in the 1930's and in 1940 to form the reservoir (Talbot & Whiteman, 2000).

A1.7 RED TARN

Red Tarn is located on Helvellyn, the third highest mountain in England, in the Ullswater catchment. It is a small lake with a surface area of < 0.1 km^2, a maximum depth of 25 m, it is at an altitude of 718 m. In the last century a dam was built to supply the mines at Glenridding (Davies & Holman, 2008).

A1.8 ULLSWATER

Ullswater is the second largest lake in the Lake District, it has a surface area of 8.9 km^2, a maximum depth of 63 m, and it is at an altitude of 145 m. Ullswater has been considered mesotrophic (Mubamba, 1989), but may now be oligotrophic (Bernhardt *et al,*. 2008). The geology of the northern end of Ullswater begins with Mell Fell Conglomerate, the middle is low lying Skiddaw slate and the south is mountainous Borrowdale volcanic rock (Talbot & Whiteman, 2000). Ullswater has several islands, the largest of which is Norfolk Island (Ellison & Cooper, 1964, 1965). Water is pumped from Ullswater to Haweswater to supplement the reservoir (Davies & Holman, 2008). Arctic charr used to be present, but have become extirpated (Maitland *et al,*. 2007).

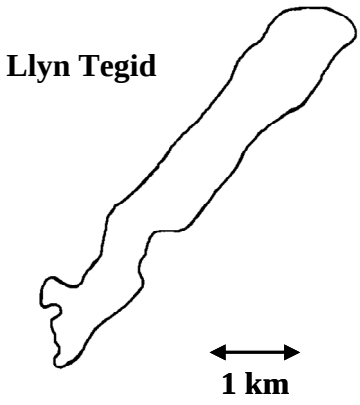

Figure A2.4 The sites sampled for *C. lavaretus* in this study: Welsh native population, Llyn Tegid

A1.9 LLYN TEGID

Llyn Tegid (sometimes referred to as Bala Lake) has a surface area of 4.1 km^2, a maximum depth of 42 m and is at an altitude of 170 m. The lake is mesotrophic but has shown some

signs of eutrophy (Happey-Wood, 2006). This is one of the British whitefish lakes that have been invaded by ruffe, probably introduced by coarse fish anglers (Winfield *et al*,. 1996, 2007b), there are also a number of other invasive fish species present (Andrews, 1977; Grainger, 1979).

Appendix 3 CATCH COMPOSITION OF NETTING ON POWAN SPAWNING GROUNDS OF LOCH LOMOND AND LOCH ECK WINTER 2005/06.

A2.1 INTRODUCTION

Loch Lomond has an extremely diverse fish community (Adams, 1994). Loch Eck has a less species-rich but equally unique fish fauna (McCarthy & Waldron, 2000). Catch composition is not equivalent, but can represent what fish are present at a certain time and place. In this case, these fish are caught alongside powan (*Coregonus lavaretus*), on and around powan spawning grounds at spawning time. Thus while powan should be the major part of the catch, fish caught in large numbers at this time may be having an impact on the spawning of powan. Differences between areas within a site may also have an impact on the species composition, such as the distinct nature of different part of Loch Lomond (Appendix 1). Changes in catch composition over time imply changes in the fish community.

A2.2 METHODS

Multi-panel Nordic-pattern benthic gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the two lochs over the winter of 2005/06. Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986). In total, 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (from 9 November 2005 to 24 January 2006), six gill nets were set overnight in Loch Eck (from 9 January 2006 to 10 January 2006). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds. Nets set outwith the month when the most powan were caught (between 30/12/05 and 24/1/06) were excluded. Nets set at sites where no powan were ever caught were excluded from analysis of catch composition.

A2.3 RESULTS

Loch Lomond and Loch Eck had only two fish in common caught on the spawning grounds: powan and brown trout. While powan is most abundant on the spawning grounds in Loch Eck, this is not the case for any area of Loch Lomond (Fig. A2.1. and A2.2.). The catch was composed of 337 powan (Loch Lomond n = 116, Loch Eck n = 223) and 47 brown trout (Loch Lomond n = 24, Loch Eck n = 23). One Arctic charr was caught in Loch Eck. All other fish were only caught in Loch Lomond (ruffe n = 266, roach n = 103, perch

n = 23, pike n = 17). One minnow, one salmon and one eel were also caught in Loch Lomond.

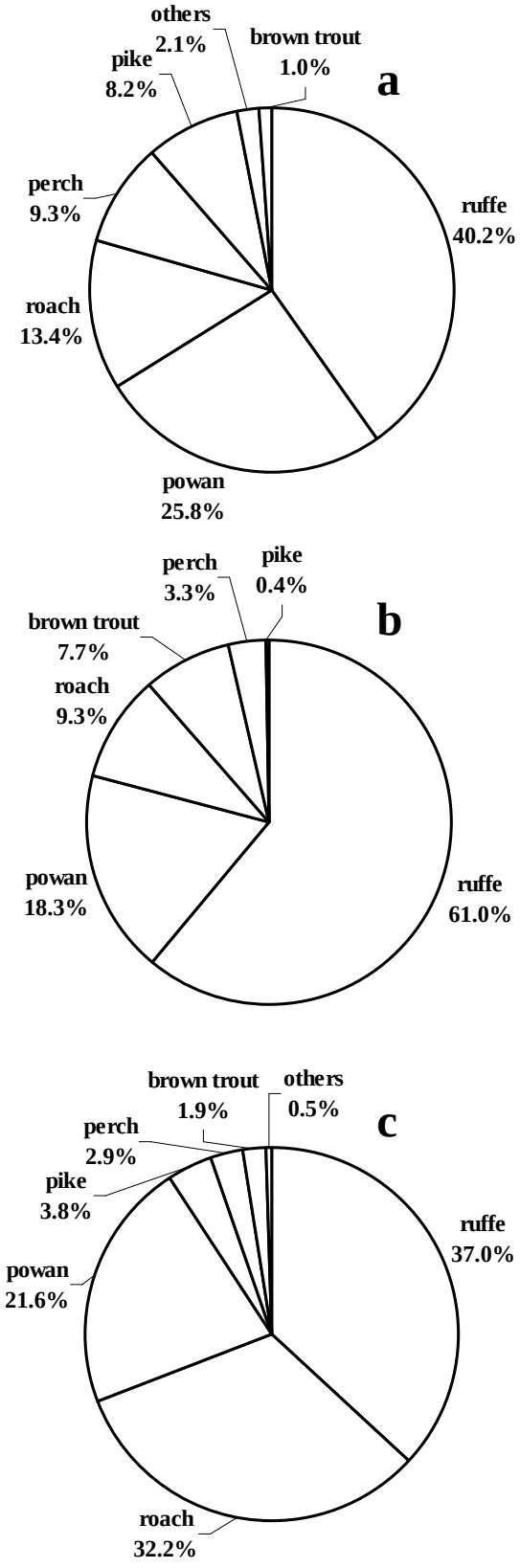

Figure A3.1 The catch composition on powan spawning grounds in the north (n = 97) (a), mid (n = 246) (b) and south (n = 208) (c) basins of Loch Lomond

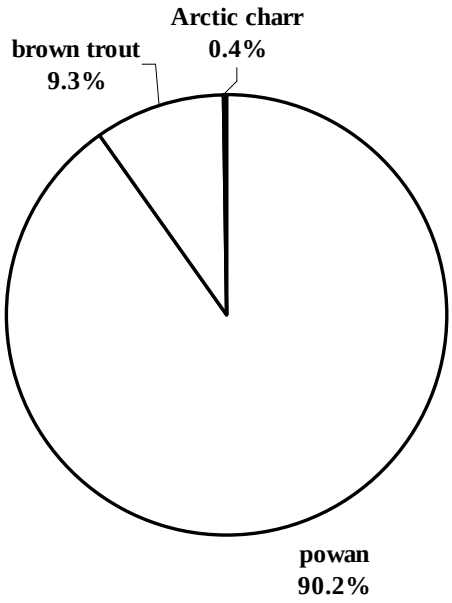

Figure A3.2 The catch composition on powan spawning grounds in Loch Eck (n = 247).

A2.4 DISCUSSION

It was found that the catch composition of fish caught alongside powan in loch Lomond is very different in each basin. The three most common fish in all the basins are ruffe, powan and roach. However, the north basin has relatively high numbers of pike and perch, perhaps suggesting that there may be high levels of predation on adult powan. The mid basin has almost half again the number of ruffe as any other basin which suggests high mortality of eggs (Chapter 6). While the south basin has more roach than powan, roach probably compete with powan for food resources. However, it is difficult to draw conclusions from this temporally isolated data, but it certainly suggests that regular catch composition data should be collected.

There is some historical information available about the relative abundances of various fish in Loch Lomond, however, Loch Eck is less well studied and little information is available. It is apparent that there have been extensive changes in the Loch Lomond fish community over time.due to a series of fish introductions (Adams *et al.*, 1990; Adams & Maitland, 1991; Adams & Mitchell, 1992; Adams, 1994; Etheridge & Adams, 2008). In the past powan was the most numerous fish in Loch Lomond (Slack *et al*, 1957). Since the introduction ruffe (*Gymnocephalus cernuus*) this fish has formed an increasingly large part of the fish community. This was the second or third most abundant fish in a series of gill netting by Adams & Tippet (1990), although powan was still the most abundant fish. In

Loch Eck there is no evidence of a decline in powan, but there appears to be a decline of Arctic charr (*Salvelinus alpinus*) (Winfield *et al.*, 2009).

The biotic environment including co-existing fish species can have large impacts on a species. How powan will respond to these changes in competition and predation (i.e. Chapter 6) over the long term remains to be seen. Nevertheless ecological changes in these populations are possible as demonstrated in Chapter 4, and should continue to be investigated e.g. Chapter 3 and Appendix 5.

Appendix 4 STABLE ISOTOPE ANALYSIS OF LOCH LOMOND FISH

A3.1 INTRODUCTION

Loch Lomond is the most species rich of all the Scottish water-bodies (Adams, 1994) and thus also has biotic interactions that are unique. These include the interaction of resident river lamprey (*Lampetra fluviatilis*) feeding on powan (*Coregonus lavaretus*) (Maitland, 1980; Adams *et al.*, 2008) and the potential impacts of numerous invasive species on the food web. Using fish captured during this study, the long-term feeding of several fish species of Loch Lomond was investigated using stable isotope analysis.

Stable isotopes of nitrogen and carbon are widely used in the study of trophic interactions (Grey 2001; Grey *et al.*, 2002). Naturally occurring stable isotopes are assimilated by animals and fractionation by biochemical processes causes the heavier isotope to be accumulated in animal tissue. $\delta^{15}N$ (the change in the ratio of ^{15}N to ^{14}N compared with a standard) is typically enriched by ca. 3-5 ‰, allowing the long-term trophic position of consumers to be estimated (Peterson & Fry, 1987; Post, 2002; Sweeting *et al.*, 2007). In contrast, trophic enrichment in $\delta^{13}C$ (the change in the ratio of ^{13}C to ^{12}C compared with a standard) is typically minor (ca. < 1 ‰) and $\delta^{13}C$ is used as a robust and consistent indicator of the carbon source the organism has been assimilating (Peterson & Fry, 1987; Hobson, 1999). Enriched $\delta^{13}C$ values are indicative of atmospheric C, while depleted values are indicative of endogenous C sources.

A3.2 METHODS

A3.2.1 Fish collection

Multi-panel Nordic-pattern benthic gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set over the winter of 2005/06. In total, 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (from 9 November 2005 to 24 January 2006). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds of powan. In total 952 fish of 10 species were caught, these were frozen within four hours of capture.

A3.2.2 Stable isotope analysis

In the laboratory, fish were defrosted, and fork length (L_f) was measured to the nearest mm. A small piece of white muscle posterior to the head and above the lateral line was removed for stable isotope analysis from 268 fish. Tissue was dried at constant temperature

(50°C for at least 48 hours), ground to a fine powder using a grinder (Revel Ltd.) and 0.5 mg of dried ground muscle was packed into pressed 10x10mm tin cups and used in simultaneous analysis of stable C and N isotopes. Stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry at the Max Planck Institute for Limnology, Germany. Stable isotope ratios are given using the δ notation expressed in units per mil where δ (‰) = [(R sample/R standard)-1] x 1000, and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The reference materials used were secondary standards of known relation to the international standards of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Typical precision for a single analysis was ± 0.1 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. Comparisons of mean C:N values indicated little need for adjustment of $\delta^{13}C$ values due to variation in lipid concentrations (Kiljunen *et al.*, 2006).

A3.3 RESULTS

Powan muscle had a mean $\delta^{13}C$ value of -27.3 ‰ ± 0.04 SE and a mean $\delta^{15}N$ value of 11.0 ‰ ± 0.07 SE. Perch (*Perca fluviatilis)* muscle had a mean $\delta^{13}C$ value of -26.2 ‰ ± 0.19 SE and a mean $\delta^{15}N$ value of 11.3 ‰ ± 0.19 SE. Roach (*Rutilus rutilus*) muscle had a mean $\delta^{13}C$ value of -25.4 ‰ ± 0.20 SE and a mean $\delta^{15}N$ value of 10.6 ‰ ± 0.17 SE. Ruffe (*Gymnocephalus cernuus*) muscle had a mean $\delta^{13}C$ value of -25.4 ‰ ± 0.42 SE and a mean $\delta^{15}N$ value of 10.7 ‰ ± 0.25 SE. The muscle of the single Atlantic salmon (*Salmo salar*) captured had a mean $\delta^{13}C$ value of -19.5 ‰ and a mean $\delta^{15}N$ value of 10.5 ‰. Brown trout (*Salmo trutta*) muscle had a mean $\delta^{13}C$ value of -24.9 ‰ ± 0.30 SE and a mean $\delta^{15}N$ value of 11.5 ‰ ± 0.25 SE. Pike (*Esox lucius*) muscle had a mean $\delta^{13}C$ value of -25.4 ‰ ± 0.48 SE and a mean $\delta^{15}N$ value of 10.7 ‰ ± 0.73 SE. The muscle of the single flounder (*Platichthys flesus*) captured had a mean $\delta^{13}C$ value of -25.3 ‰ and a mean $\delta^{15}N$ value of 11.3 ‰. The muscle of the single bream (*Abramis brama*) captured (the first confirmed in Loch Lomond, Etheridge & Adams, 2008) had a mean $\delta^{13}C$ value of -29.1 ‰ and a mean $\delta^{15}N$ value of 11.0 ‰. Eel (*Anguilla anguilla*) muscle had a mean $\delta^{13}C$ value of -25.8 ‰ ± 0.33 SE and a mean $\delta^{15}N$ value of 12.0 ‰ ± 0.31 SE (Fig. A3.1.).

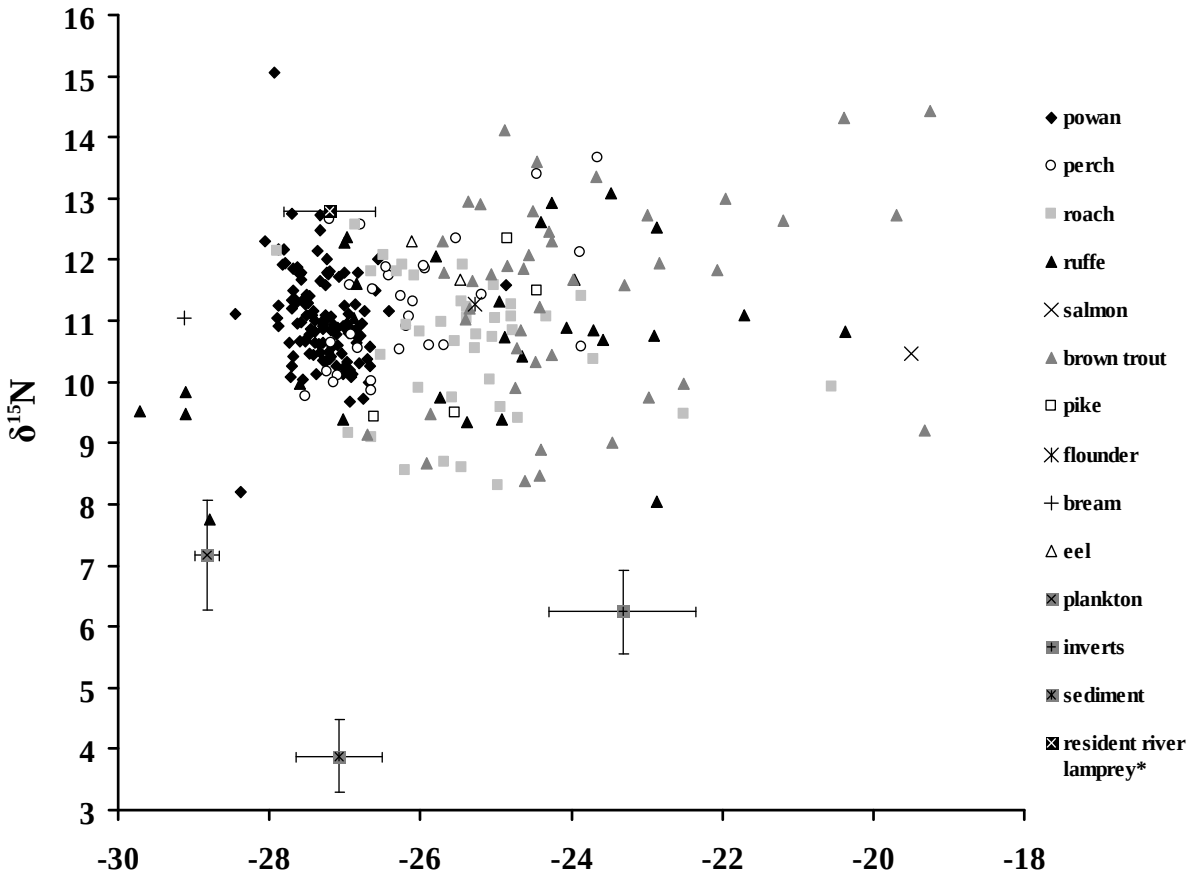

Figure A4.1 Variation in $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue from fish from Loch Lomond. Mean (± S.E.) stable isotope values of plankton, benthic invertebrates and sediment are also included. The mean (± S.E.) stable isotope values resident river lamprey* have been extracted from Adams *et al.* (2008). This is able to demonstrate the close association of powan and resident river lamprey.

A3.4 DISCUSSION

These results give an indication as to the complexity of the trophic ecology of Loch Lomond. A marine influence (high $\delta^{13}C$ value) is apparent in the single Atlantic salmon captured over this series of netting and in some brown trout. However, many of the brown trout seem to exhibit a $\delta^{13}C$ value intermediate to a fully marine or fully freshwater signature as discussed in Chapter 7. Non-native ruffe have a wider trophic niche than any other species found in Loch Lomond. This confirms the reputation of ruffe as an ideal invader species (see Chapter 6), able to adapt and exploit almost any environment and resource. It appears from the results that ruffe trophically interact with many species in Loch Lomond, having potential be predator, prey and competitor to a species. Individual powan are closely grouped together, feeding on much the same items and thus gaining their C from a similar source. There is some variation between powan; however, this is most likely due to some basin-specific feeding fidelity rather than a variation in diet (see

Chapter 5). However, the interation between the freshwater-feeding resident river lamprey (*Lampetra fluviatilis*) morph (data extracted from Adams *et al*., 2008). and powan is very clear in these results, these lamprey appear from these results to feed on mainly on powan, as have been indicated by other authors (Maitland, 1980; Maitland *et al*., 1994). It remains to be seen what the long-term effect of a reduction of powan numbers (see Chapter 3) might have on these lamprey, whether for instance they might broaden their food base, or perhaps decline in numbers. This information however, reiterates that the conservation and ecology of a species can never be considered in complete isolation to co-existing and potentially interacting species. In the future, this data might be grounded by examining the stomach contents of Loch Lomond fish in addition to the stable isotope analysis.

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Appendix 5 STABLE ISOTOPE ANALYSIS OF MUSCLE TISSUE OF POWAN FROM LOCH LOMOND, LOCH ECK, LOCH SLOY AND CARRON VALLEY RESERVOIR.

A4.1 INTRODUCTION

Trophic ecology is particularly interesting in postglacial fishes which show great phenotypic variation, since differences in trophic ecology is often associated with differences in phenotype (Schluter, 1995; Amundsen *et al.*, 2004; Knudsen *et al.*, 2007). This can be due to genetic adaptation and phenotypic plasticity. There are four populations of powan (*Coregonus lavaretus*) in Scotland. Two of these Loch Lomond and Loch Eck are native populations located in different catchments and have been separated for thousands of years. The two refuge populations were established using Loch Lomond powan between 1988 and 1991 (Maitland & Lyle, 1992; 1995). and thus have only been separated from each other and Loch Lomond for a short period of time. Stable isotope analysis is used to assess the long term trophic ecology of these populations.

Stable isotopes of nitrogen and carbon are widely used in the study of trophic interactions (Grey 2001; Grey *et al.*, 2002). Naturally occurring stable isotopes are assimilated by animals and fractionation by biochemical processes causes the heavier isotope to be accumulated in animal tissue. $\delta^{15}N$ (the change in the ratio of ^{15}N to ^{14}N compared with a standard) is typically enriched by ca. 3-5 ‰, allowing the long-term trophic position of consumers to be estimated (Peterson & Fry, 1987; Post, 2002; Sweeting *et al.*, 2007). In contrast, trophic enrichment in $\delta^{13}C$ (the change in the ratio of ^{13}C to ^{12}C compared with a standard) is typically minor (ca. < 1 ‰) and $\delta^{13}C$ is used as a robust and consistent indicator of the carbon source the organism has been assimilating (Peterson & Fry, 1987; Hobson, 1999).

A4.2 METHODS

A4.2.1 Fish collection

Multi-panel Nordic-pattern benthic gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the sites over the winter of 2005/06. Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986). In total, 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (from 9 November 2005 to 24 January 2006), six gill nets were set overnight in Loch Eck (from 9 January 2006 to 10 January 2006), seven gill nets, were set overnight in Loch Sloy (from 21 December 2005 to 28 December 2005) and ten gill nets

were set overnight in Carron Valley Reservoir (from 3 January 2006 to 5 January 2006) (for details of sites, see Appendix 1). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds. During this period a total of 341 powan were caught (Loch Lomond n = 118 and Loch Eck n = 223, Loch Sloy n = 76 and Carron Valley Reservoir n = 58). Fish were frozen within four hours of capture.

A4.2.2 Stable isotope analysis

In the laboratory, fish were defrosted, and fork length (L_f) was measured to the nearest mm. A small piece of white muscle posterior to the head and above the lateral line was removed for stable isotope analysis from 307 fish. Tissue was dried at constant temperature (50°C for at least 48 hours), ground to a fine powder using a grinder (Revel Ltd.) and 0.5 mg of dried ground muscle was packed into pressed 10x10mm tin cups and used in simultaneous analysis of stable C and N isotopes. Stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry at the Max Planck Institute for Limnology, Germany. Stable isotope ratios are given using the δ notation expressed in units per mil where δ (‰) = [(R sample/R standard)-1] x 1000, and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The reference materials used were secondary standards of known relation to the international standards of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Typical precision for a single analysis was ± 0.1 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. Comparisons of mean C:N values indicated little need for adjustment of $\delta^{13}C$ values due to variation in lipid concentrations (Kiljunen *et al.,* 2006).

A4.2.3 Invertebrate and sediment collection

A baseline isotope value was required for each site to account for background isotopic differences and therefore allow comparison between powan from different sites. Despite several attempts at benthic invertebrate collection using at Eckmann grab and kick sampling there were few instances of any benthic invertebrates that were common between all sites and therefore could be used as a baseline. This would have been best achieved by collecting filter feeding bivalves (pelagic signature) and algal scraping snails (benthic signature). It was decided that the use of plankton was problematic due to their fast turnover of stable isotopes. Therefore, sediment samples were taken from each site to provide this baseline. This was processed as above, but at Strathclyde University. The value of $\delta^{13}C$ and $\delta^{15}N$ of the sediment from a site was taken away from the value of $\delta^{13}C$ and $\delta^{15}N$ of the muscle tissue of powan at that site resulting in adjusted values.

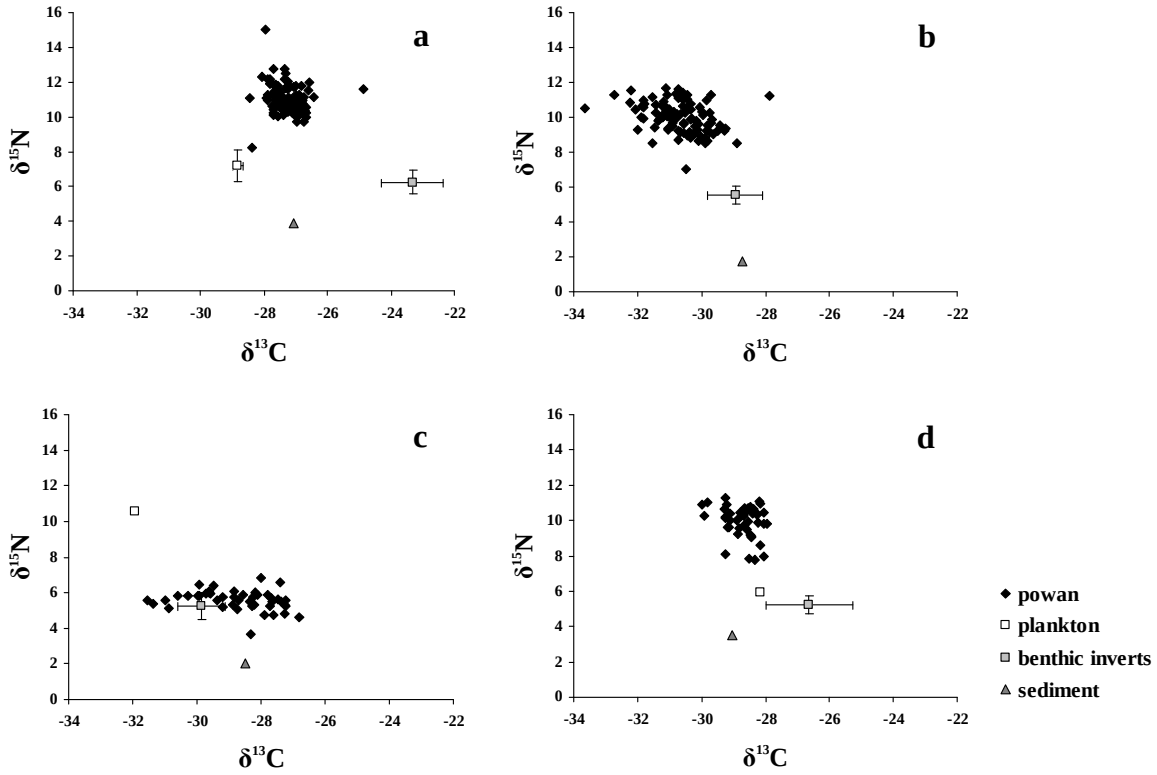

Figure A5.1 Variation in $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue from powan and of plankton (mean ± SE only at Loch Lomond), benthic invertebrates (mean ± S.E.) and sediment samples from Loch Lomond (a), Loch Eck (b), Loch Sloy (c) and Carron Valley Reservoir (d).

A4.3 RESULTS

Loch Lomond powan muscle had a mean $\delta^{13}C$ value of -27.3 ‰ ± 0.04 SE and a mean $\delta^{15}N$ value of 11.0 ‰ ± 0.07 SE (Fig. A4.1.). Plankton from this site had a mean $\delta^{13}C$ value of -28.8 ‰ ± 0.16 SE and a mean $\delta^{15}N$ value of 7.2 ‰ ± 0.90 SE. Macroinvertebrates had a mean $\delta^{13}C$ value of -23.3 ‰ ± 0.97 SE and a mean $\delta^{15}N$ value of 6.2 ‰ ± 0.69 SE. A single sample of sediment from this site had a mean $\delta^{13}C$ value of -27.1 ‰ and a mean $\delta^{15}N$ value of 3.9 ‰.

Loch Eck powan muscle had a mean $\delta^{13}C$ value of -30.7 ‰ ± 0.09 SE and a mean $\delta^{15}N$ value of 10.0 ‰ ± 0.09 SE (Fig. A4.1.). Macroinvertebrates had a mean $\delta^{13}C$ value of -28.9 ‰ ± 0.87 SE and a mean $\delta^{15}N$ value of 5.5 ‰ ± 0.52 SE. A single sample of sediment from this site had a mean $\delta^{13}C$ value of -28.9 ‰ and a mean $\delta^{15}N$ value of 1.8 ‰.

Loch Sloy powan muscle had a mean $\delta^{13}C$ value of -28.8 ‰ ± 0.18 SE and a mean $\delta^{15}N$ value of 5.6 ‰ ± 0.08 SE (Fig. A4.1.). A single sample of plankton from this site had a mean $\delta^{13}C$ value of -31.9 ‰ and a mean $\delta^{15}N$ value of 10.6 ‰. Macroinvertebrates had a

mean $\delta^{13}C$ value of -29.9 ‰ ± 0.74 SE and a mean $\delta^{15}N$ value of 5.2 ‰ ± 0.76 SE. A single sample of sediment from this site had a mean $\delta^{13}C$ value of -28.5 ‰ and a mean $\delta^{15}N$ value of 2.0 ‰.

Carron Valley Reservoir powan muscle had a mean $\delta^{13}C$ value of -28.7 ‰ ± 0.07 SE and a mean $\delta^{15}N$ value of 10.0 ‰ ± 0.12 SE (Fig. A4.1.). A single sample of plankton from this site had a mean $\delta^{13}C$ value of -28.2 ‰ and a mean $\delta^{15}N$ value of 5.9 ‰. Macroinvertebrates had a mean $\delta^{13}C$ value of -26.6 ‰ ± 1.4 SE and a mean $\delta^{15}N$ value of 5.2 ‰ ± 0.51 SE. A single sample of sediment from this site had a mean $\delta^{13}C$ value of -29.0 ‰ and a mean $\delta^{15}N$ value of 3.5 ‰.

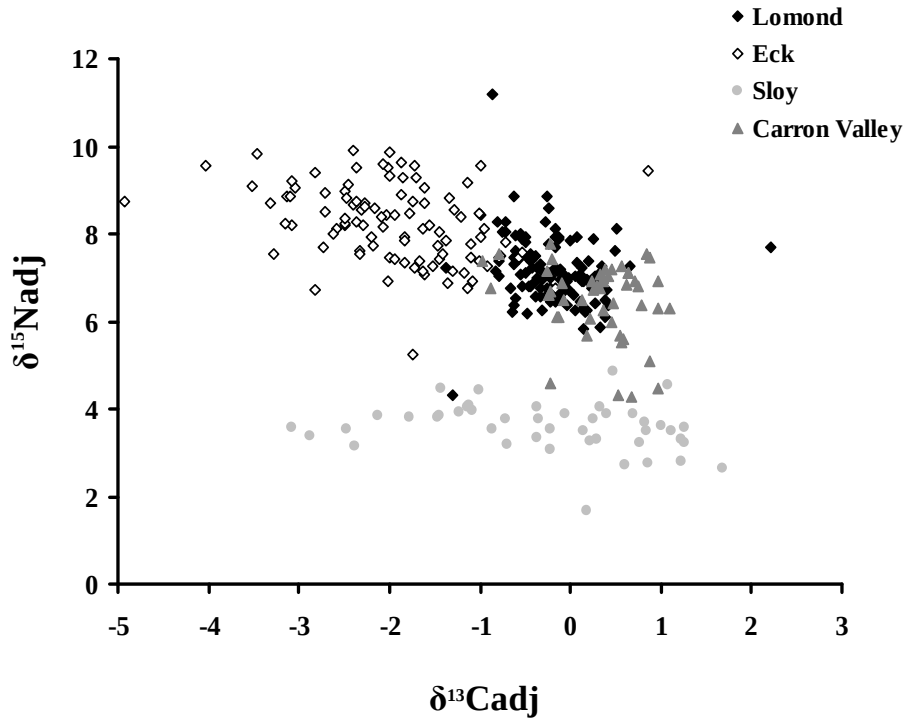

Figure A5.2 $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue from powan from Loch Lomond, Loch Eck, Loch Sloy and Carron Valley Reservoir, adjusted using sediment stable isotope values from the site of origin.

To compare between sites the background differences in nutrients was taken into account by using the sediment $\delta^{13}C$ and $\delta^{15}N$ values as a baseline to create adjusted isotopic values of powan muscle tissues (Fig. A4.2). There were significant differences between $\delta^{13}C$adj (ANOVA, $F_{3,303} = 141.7$, $p < 0.0001$) and $\delta^{15}N$adj (ANOVA, $F_{3,303} = 358.1$, $p < 0.0001$) of powan muscle between sites. Bonferroni *post hoc* testing found significant differences in $\delta^{13}C$adj of powan muscle between all pairwise comparisons ($p < 0.01$) apart from Lomond and Sloy ($p > 0.99$) and significant differences in $\delta^{15}N$adj of powan muscle between all

pairwise comparisons ($p < 0.0001$). After adjustment, the site in which the powan are most enriched in $\delta^{13}C$ is Carron Valley (indicative of atmospheric C), while powan from Eck are most depleted (indicative of endogenous C sources). After adjustment, the site in which the powan are most enriched in $\delta^{15}N$ is Loch Eck (higher trophic level), while powan from Loch Sloy are most depleted (lower trophic level).

There were some significant correlations between fork length (L_f) and stable isotope values of powan muscle tissue. There were significant positive correlations between L_f and δ^{13}Cadj in powan from Loch Lomond (Pearson correlation = 0.28, $p < 0.01$), Loch Eck (Pearson correlation = 0.27, $p < 0.01$), and Carron Valley Reservoir (Pearson correlation = 0.48, $p < 0.001$), but there was a significant negative correlation in powan from Loch Sloy (Pearson correlation = -0.63, $p < 0.0001$) (Fig. A4.3.). There were significant negative correlations between L_f and δ^{15}Nadj in powan only from Loch Lomond (Pearson correlation = -0.37, $p < 0.0001$) and Loch Eck (Pearson correlation = -0.37, $p < 0.001$), however, these were the only sites from which very small powan were caught and this pattern is indicative of an ontogenetic shift in feeding (Fig. A4.4.).

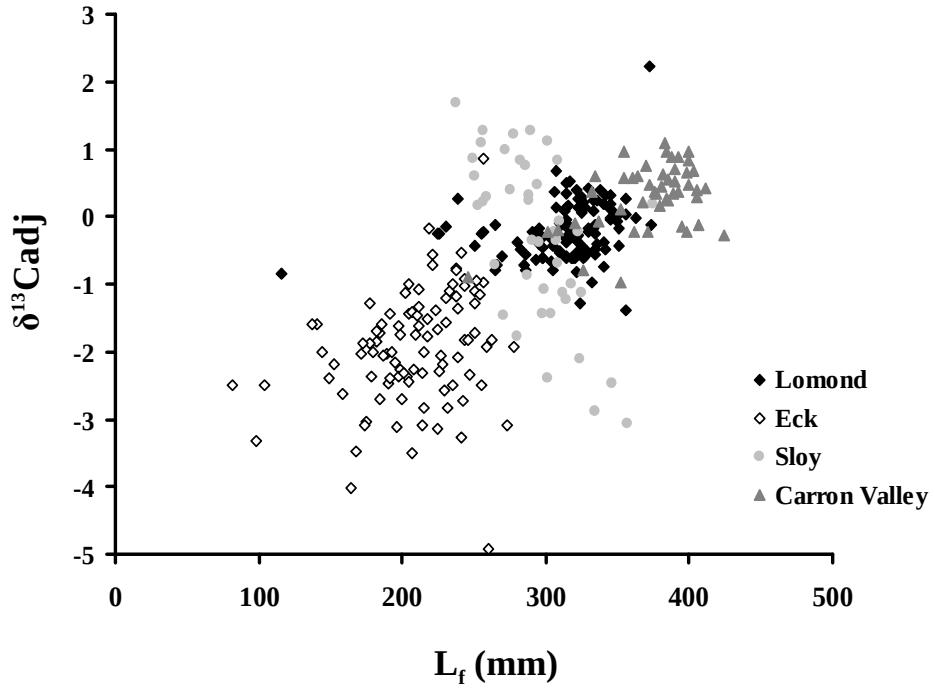

Figure A5.3 L_f and $\delta^{13}C$ of muscle tissue from powan from Loch Lomond, Loch Eck, Loch Sloy and Carron Valley Reservoir, adjusted using sediment stable isotope values from the site of origin.

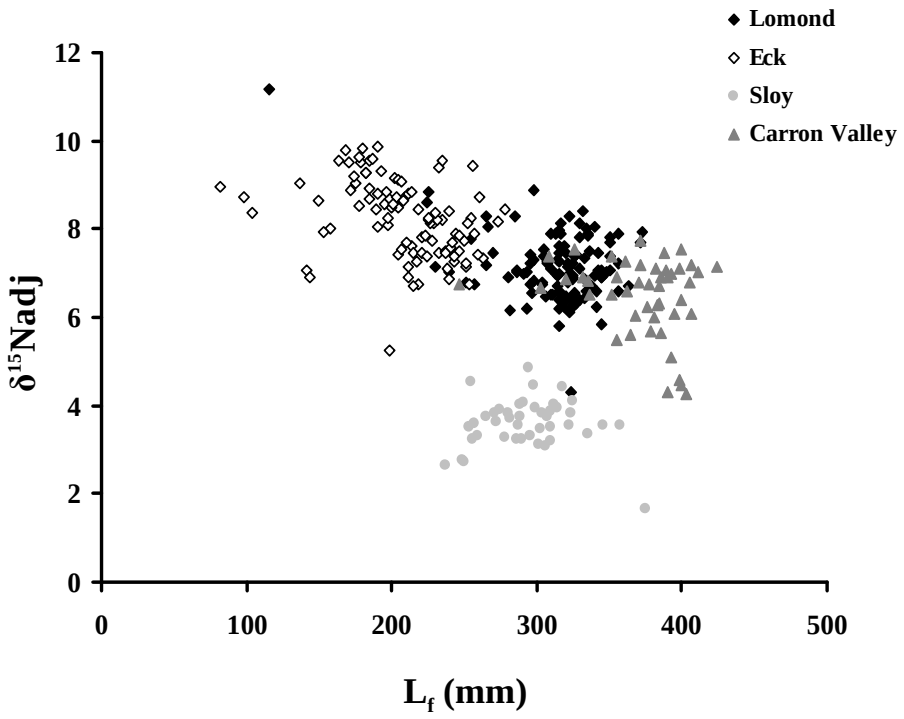

Figure A5.4 L_f and $\delta^{15}N$ of muscle tissue from powan from Loch Lomond, Loch Eck, Loch Sloy and Carron Valley Reservoir, adjusted using sediment stable isotope values from the site of origin

A4.4 DISCUSSION

It was found that there are significant differences in long term trophic ecology between populatons of powan in Scotland. The results suggest powan from Loch Lomond appeared to be mainly obtaining their C from a similar source to each other, possibly one source. However, powan from Loch Eck were relatively spread out along the $\delta^{13}C$ axis and so the population as a whole appears to have a diet that encompasses C from more than one source. Individuals may be feeding long-term on different mixtures of food that contains C from different sources. This has been suggested in previous studied reviewed in Chapter 3. These have found that Loch Lomond powan tend to feed more on plankton, while Loch Eck powan tend to feed on a mixture of benthic invertebrates and plankton (Pomeroy, 1991, 1994). It is likely that these long term feeding differences have had a role in some of the trophically-linked phenotype differences between these populations e.g. head shape (Chapter 3).

Powan from Loch Sloy were very spread out in $\delta^{13}C$ and individuals appear to have diets that vary from each other and which encompasses C from more than one source. They also had a very low $\delta^{15}N$ value, this may be due to differences in the background nutrient levels

at this site, although macroinvertebrates and plankton are not trophically below Loch Sloy powan as would be expected. Moreover even when corrected for nutrient differences, this pattern is maintained. The results for muscle tissue of powan at Carron Valley Reservoir suggested that fish from this site had a very similar diet to one another. While this may be indicative of a diet that encompasses C from only one source, a study by Deverill (2000) found that Carron Valley Reservoir powan feed on a mixture of planktonic and benthic prey. Therefore it appears that powan from this site may have a mixed diet, but that it is very similar between individuals over the long term. It is likely that these long term feeding differences have had a role in some of the trophically-linked phenotype differences e.g. head shape (Chapter 4) between these populations, and between these populations and the donor population of Loch Lomond.

Apart from those orininating in Loch Sloy there was a general trend that larger powan had a more enriched $\delta^{13}C$ signature, indicative of atmospheric C. While in the populations in which younger fish were caught there is evidence of an ontogenetic trophic shift in $\delta^{15}N$ value. Generally the Loch Sloy powan appear to have a very different long term trophic ecology to all the other Scottish populations, native and refuge. However, it is difficult to ground this data without also examining stomach contents and it is suggested that this would be the next step in examination of powan ecology.

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Appendix 6 STATIC LIFE TABLE OF LOCH LOMOND POWAN

A5.1 INTRODUCTION

A life table represents age-specific birth and death probabilities allowing these to be analysed. This information is important in recognising demographic changes in populations. While a dynamic life table follows individuals from birth to death, a static life table estimates the age structure of a population at one point in time. Brown *et al*. (1991) produced a static life table for Loch Lomond powan using data collected from survey netting from 1980 – 1988. In a species like powan (*Coregonus lavaretus*) which does not usually survive capture (gill netting) a static life table is necessary. Since a previous life table has been published, another one can be constructed using current data and compared in order to detect changes in demography that would suggest changes in age-specific mortality or fecundity.

A5.2 METHOD

Multi-panel Nordic-pattern benthic gill nets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the two lochs over the winter of 2005/06. Nordic nets are not selective for coregonids over the modal size range 78 mm to 613 mm fork length (L_f) (Jensen, 1986). 75 gill nets were set overnight in sites in the north, mid and south basins of Loch Lomond (from 9 November 2005 to 24 January 2006). The nets were set immediately prior to and during spawning time, on known or presumed spawning grounds. During this period a total of 118 powan were caught. Fish were frozen within four hours of capture. Fork length was measured to the nearest mm and fish were aged by scale reading.

Using the figures provided by Brown *et al.* (1991), and data from catches in this study, a life table was constructed. Below ages 6+ to 8+ the numbers of individuals were back calculated using survival proportion recorded by Brown *et al.* (1991). For ages for which there were 2 or less individuals (1+ to 3+) the mean length recorded by Brown *et al.* (1991) was used. The proportion of females reproducing, the number of oocytes per mean length female and the total number of oocytes were also taken from Brown *et al.* (1991). It was assumed that there was a sex ratio of 1:1 in this population.

A5.3 RESULTS

Total fecundity is greatest for the 3-4 age class, similar to that found in powan 1980-1987 (Brown *et al.*, 1991). At age 4-5 mean fork length is less than that found for powan 1980-1987, however mean fork length is greater than that found in powan previously in fish aged 5-6 and older (Brown *et al.*, 1991) (Fig. A5.1.).

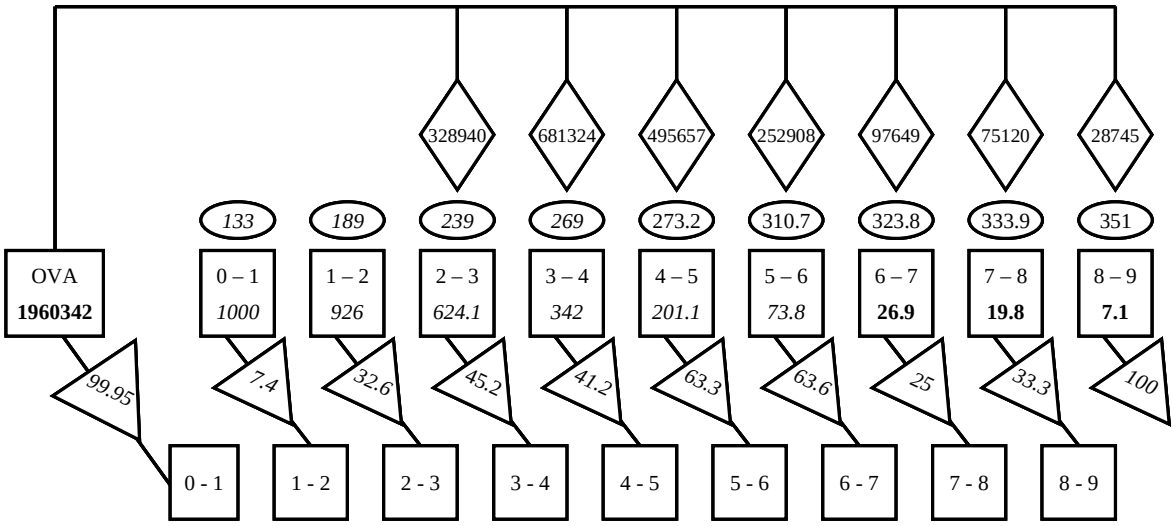

Figure A6.1 Static life table for Loch Lomond powan, caught winter 2005/06. Upper rectangles: age-group (x) and number of individuals from 1000 at age group 0-1 (italics show back calculated numbers for ages 5-6 and below; bold numbers are those calculated from this series of netting). Lower rectangles: next age group (x + 1). Ovals: mean fork length at age (italics show mean fork length taken from Brown *et al.* (1991). Diamonds: number of ova produced, calculated from Brown *et al.* (1991) for ages 3-4 and below). Age groups 0-1 and 1-2 do not reproduce

A5.4 DISCUSSION

The long-term effect of invasive species is of considerable interest, particularly as there are usually few avenues of mitigation once invasive species are established. In Loch Lomond obvious evidence of an effect on the life history of powan from the introduction of ruffe has not yet been found. This life table constructed using data collected from a limited netting in winter 2005/06, differs very little from a previous life table constructed by Brown *et al.* (1991) using data collected from survey netting from 1980 – 1988. This suggests that there have been no changes in demography and life history between these times. It is possible however, that due to the limited nature of the data used in the later life table and the fact that information from the Brown *et al.* (1991) life table was used to supplement this limited information, that any changes in demography over time may have

been obscured. The impacts of ruffe in increasing egg-stage powan mortality for example, might be responded to in egg size and number, and timing of spawning, or hatching. Certainly in other species there are examples of life history responses, such as egg incubation and emergence timing responding to high mortality, particularly in amphibians (Warkentin, 1995; Vonesh, 2005) and fish (Wedekind, 2002; Jones *et al.*, 2003; Kusch & Chivers, 2004; Wedekind & Muller, 2005; Evans *et al.*, 2007). The possible effect of this may be unpredictable and profound as changes in the life history at one life stage may have fitness consequences at another (Ojanguren *et al.*, 1996; Jones *et al.*, 2003; Kusch & Chivers, 2004). As of yet, Loch Eck has not been impacted by invasive species, however, the potential loss of Arctic charr (*Salvelinus alpinus*) from this site may result in changes in this population which is part of a unique fish community containing all native British salmonids. No life table has been constructed for this population, however, using data from previous studies this might be possible, and would be desirable to provide a baseline against which to measure possible demographic changes in the future.

The future impact of biotic changes on the native populations of powan are hard to predict, interactions between species such as predation and competition can have large, multidirectional and probably unpredictable impacts as part of a heterogeneous and non-static environment. This holds true for environmental changes. In the future climate change is also likely to effect these populations. Around Loch Lomond where much long term data is collected there have been changes in temperature regimes and biotic temporal indicators (i.e. moth emergence and disappearance) that have been linked to the effects of climate change (Krokowski, 2007; Salama *et al.*, 2007). A temperature change may affect powan directly or indirectly. An increase in temperature would likely directly affect powan adversely since whitefish are cold adapted e.g. egg mortality is positively related to temperature (Slack *et al.*, 1957; Bagenal, 1966, 1970). However, other potential impacts e.g. on zooplankton availability and timing, growth rates and hatching times of powan, may have beneficial or negative effects. Since the native Scottish powan sites are in close proximity it is likely these will both be effected by changes in climate, through due to their differing biology and population status these may respond differently to a similar pressure. If changes in ecology and life history are found in the future, it may be difficult to tease apart what are responses to biotic and/or environmental changes. There is the added complication of climate change likely increasing the invasion success and establishment of alien species (Adams, 1994). The consequences to biotic and environmental changes are thus difficult to predict, but powan are potentially vulnerable to both. All aspects of the biology of the native and refuge populations of powan should be monitored regularly. Any

information on changes in response to biotic and environmental pressures will be useful for other populations of *C. lavaretus* and other similar species.

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